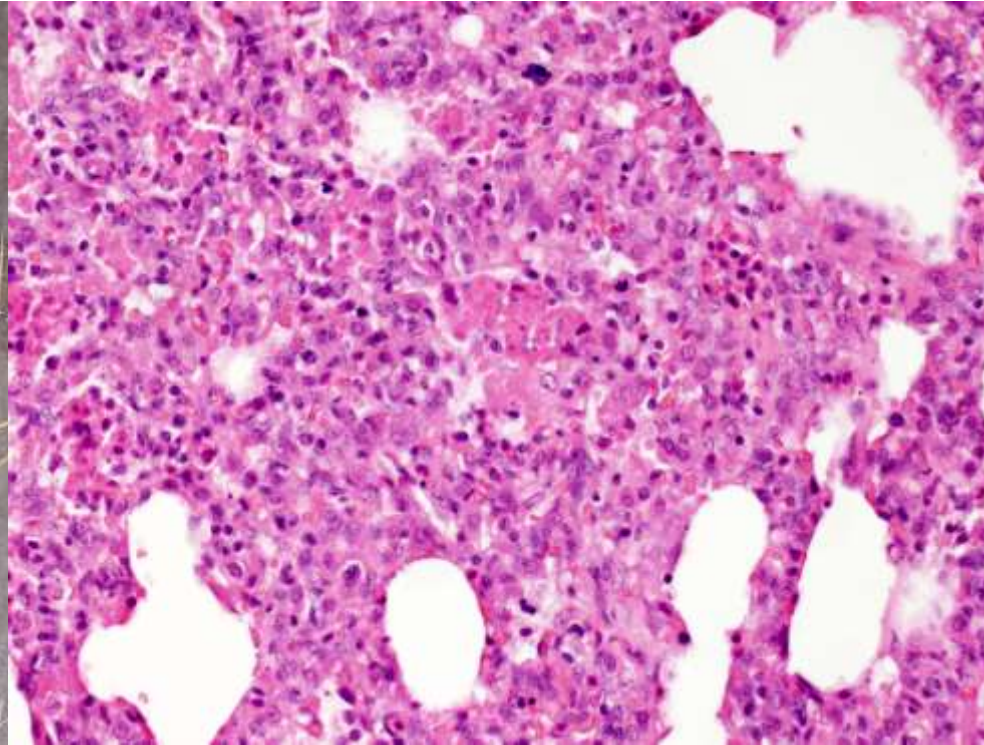




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Associate professor
Dept. of Pathology
University of Veterinary Medicine, Budapest

Jurmala, 29. March 2019

PRRS (Porcine **reproductive** and **respiratory** syndrome)



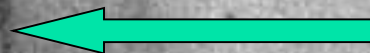
Outline

1. The virus
2. The host
3. The disease

PRRS

- 1987 USA, 1990 Germany
- 1991 Lelystad, The Netherlands (Wensvoort et al.)
- 1992 USA (Collins et al.)
- 2006 China: highly pathogenic strains, 50–100% mortality:
 - **High Fever Syndrome (PRRSV-2)**
NSP2 ~30AA deletion (not related to virulence)
 - **Denmark 2010-2011 severe reproductive disorders (PRRSV-2)**
 - **Highly Pathogenic PRRSV-1, Subtype-3**
 - Karnychuk et al. 2010: „LENA”, Van Doorselaere et al. 2012
 - Morgan et al. 2012: „SU-1bel”
 - **Highly Pathogenic PRRSV-1, Subtype-1**
 - **Belgium 2013** – 13V092 (Frydas et al. 2015) long anorexia, fever, higher replication rates in the upper respiratory track!!!
 - **Italy 2014** – PR-392014, PR-402014 (Canelli et al. 2014)
50% mortality in weaners
 - **Austria 2015** – AUT15-33 „ACRO” strain (Sinn et al 2016.) up to 90% losses among piglets, 40% in the nursery, repeat breeding etc. Similar strains were found in Germany.

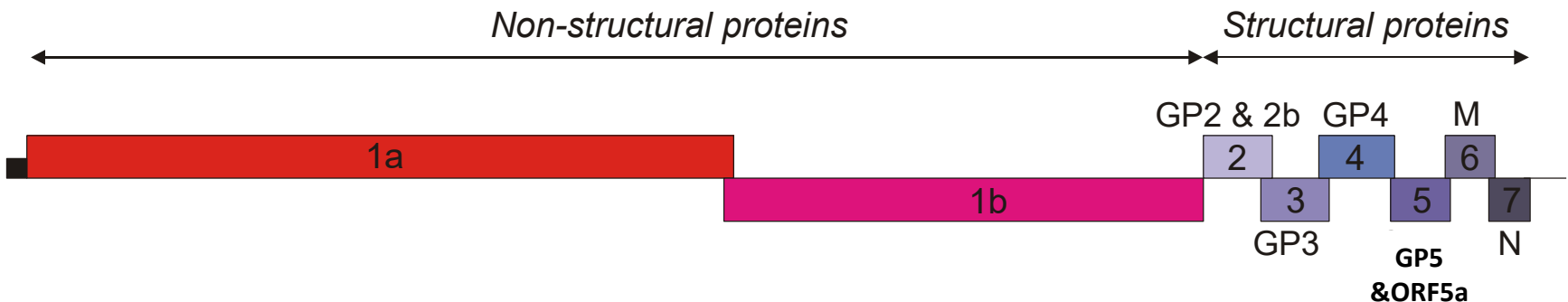
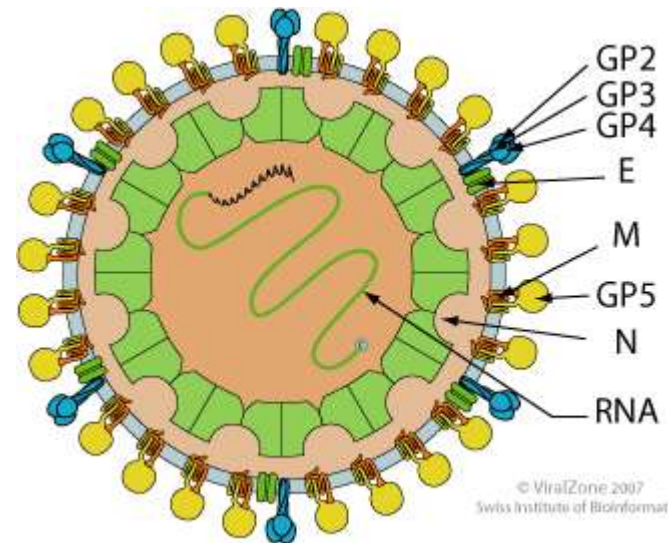
**Enveloped
50–60nm**



200 nm

PRRSV-1, PRRSV-2

- ss RNA virus, quasispecies
- *Arteriviridae* family
 - Replicates in macrophages
 - Prolonged viremia
 - Persisting (?) infection



Taxonomy ICTV

Order: Nidovirales

Suborder: Arnidoviridae

Family: Arteriviridae

Subfamily: Crocarterivirinae

Subfamily: Equarterivirinae

Subfamily: Heroarterivirinae

Subfamily: Simarterivirinae

Subfamily: Variarterivirinae

Genus: Betaarterivirus

Subgenus: Ampobartevirus

Species: Betaarterivirus suid 2
(PRRSV-2)

Subgenus: Chibartevirus

Subgenus: Eurpobartevirus

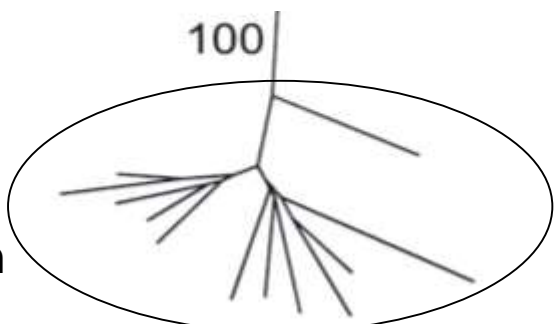
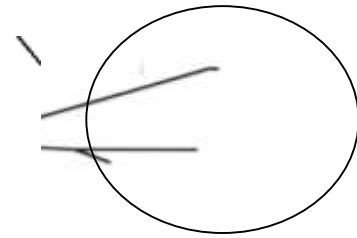
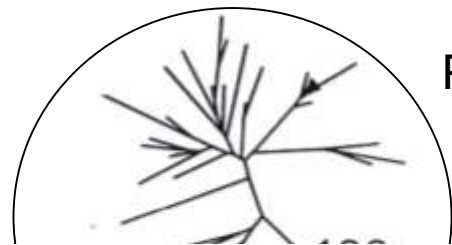
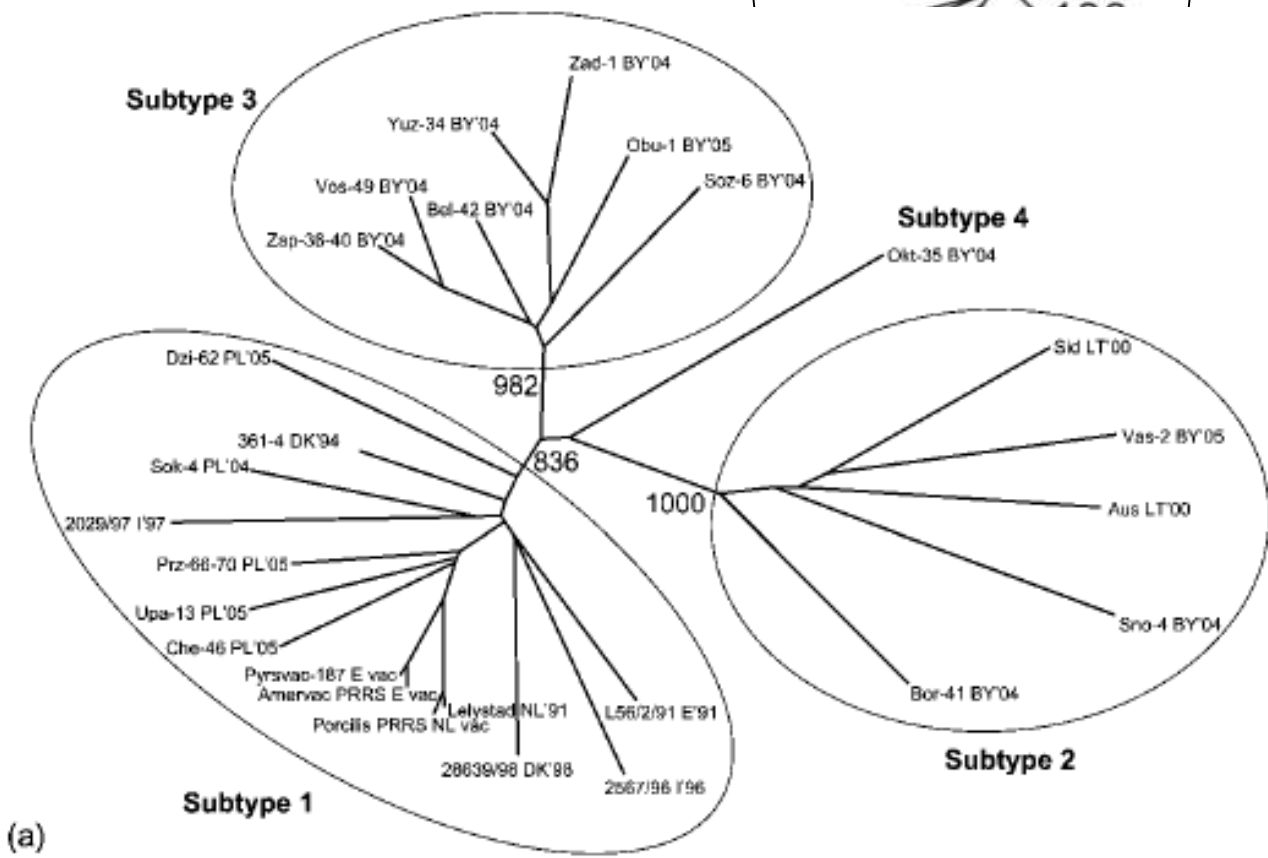
Species: Betaarterivirus suid 1
(PRRSV-1)

Genus: Gammaarterivirus

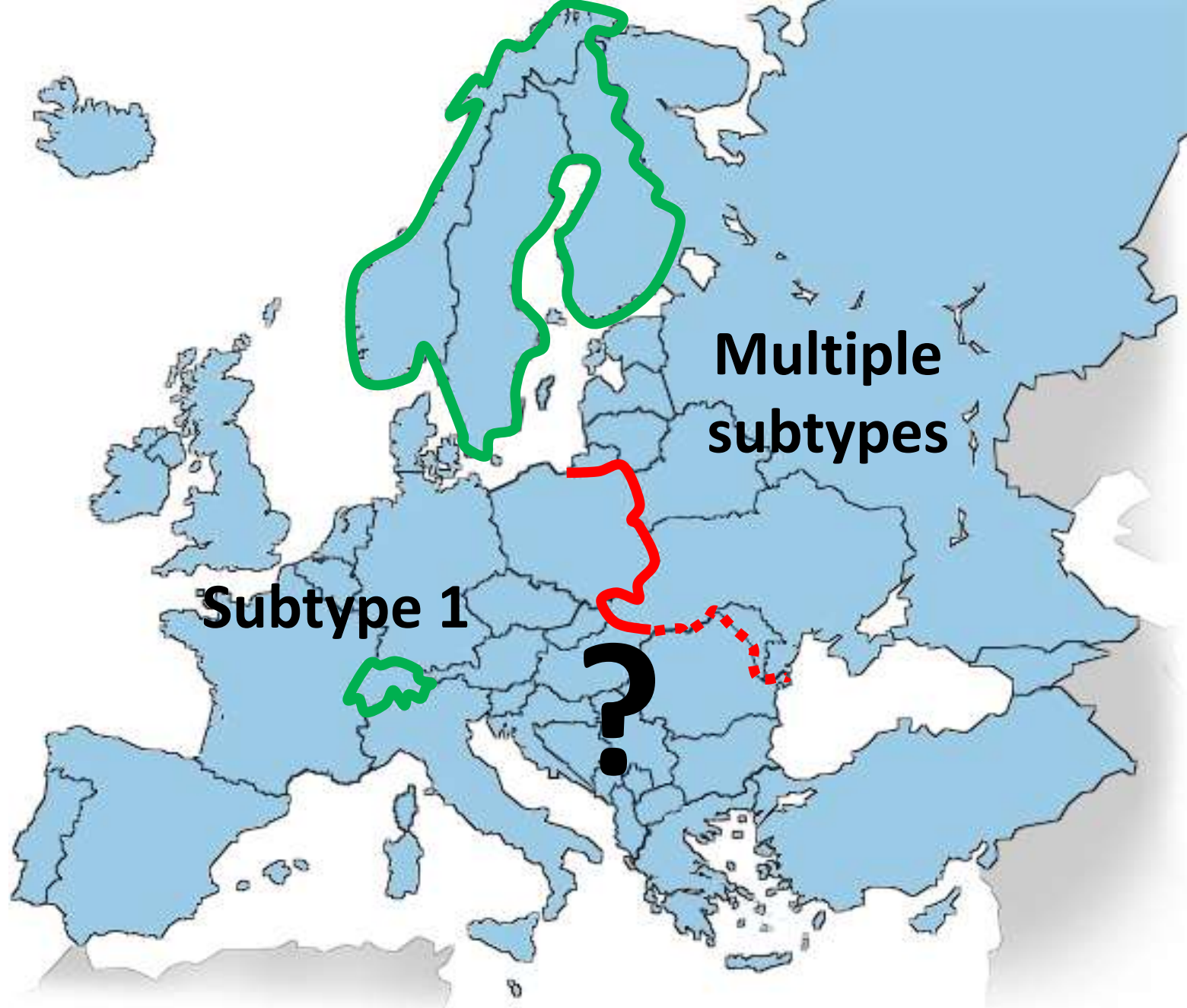
Subfamily: Zealarterivirinae

Genetic variability

- Bet
- ORI
- suri
- Eur
- 4 st



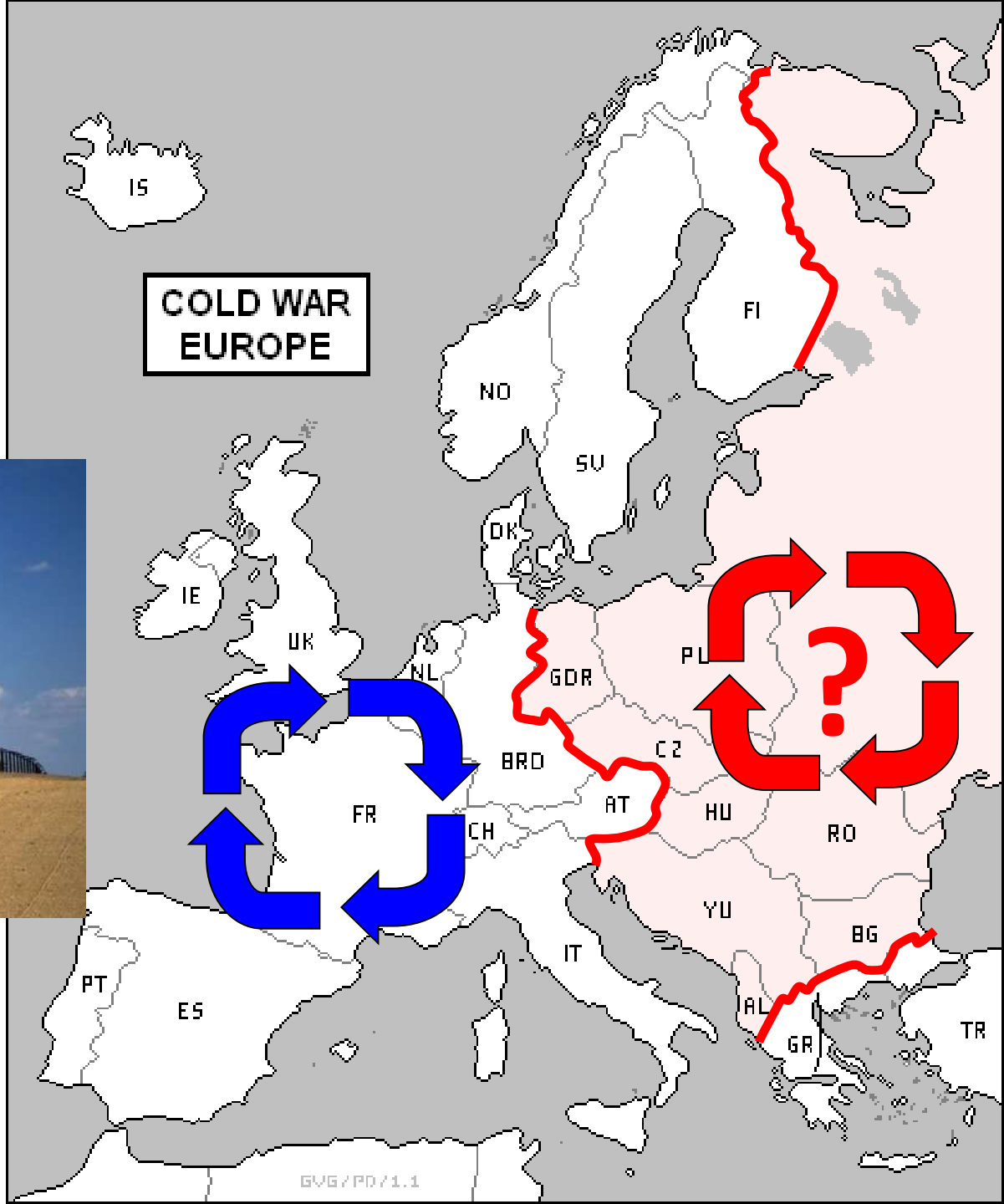
ility



Multiple subtypes

Subtype 1

?



What is the origin of PRRSV?

- Highest diversity is always found at the site of it's origin – Belarus, Russia, Baltics
- How did PRRSV come here – no/minimal live pig transport towards Western Europe
- “die Wildschwein Plage” (Thomas Fleischmann 2016)
 - Extreme wild boar population increase in the late 1970-ties culminating in 1988 in the DDR
- The first seropositive serum was from 1987 (Ohlinger et al. 2000)
- First clinical evidence of PRRS in 1990 (Lindhaus and Lindhaus 1991)

SCIENTIFIC REPORTS



OPEN

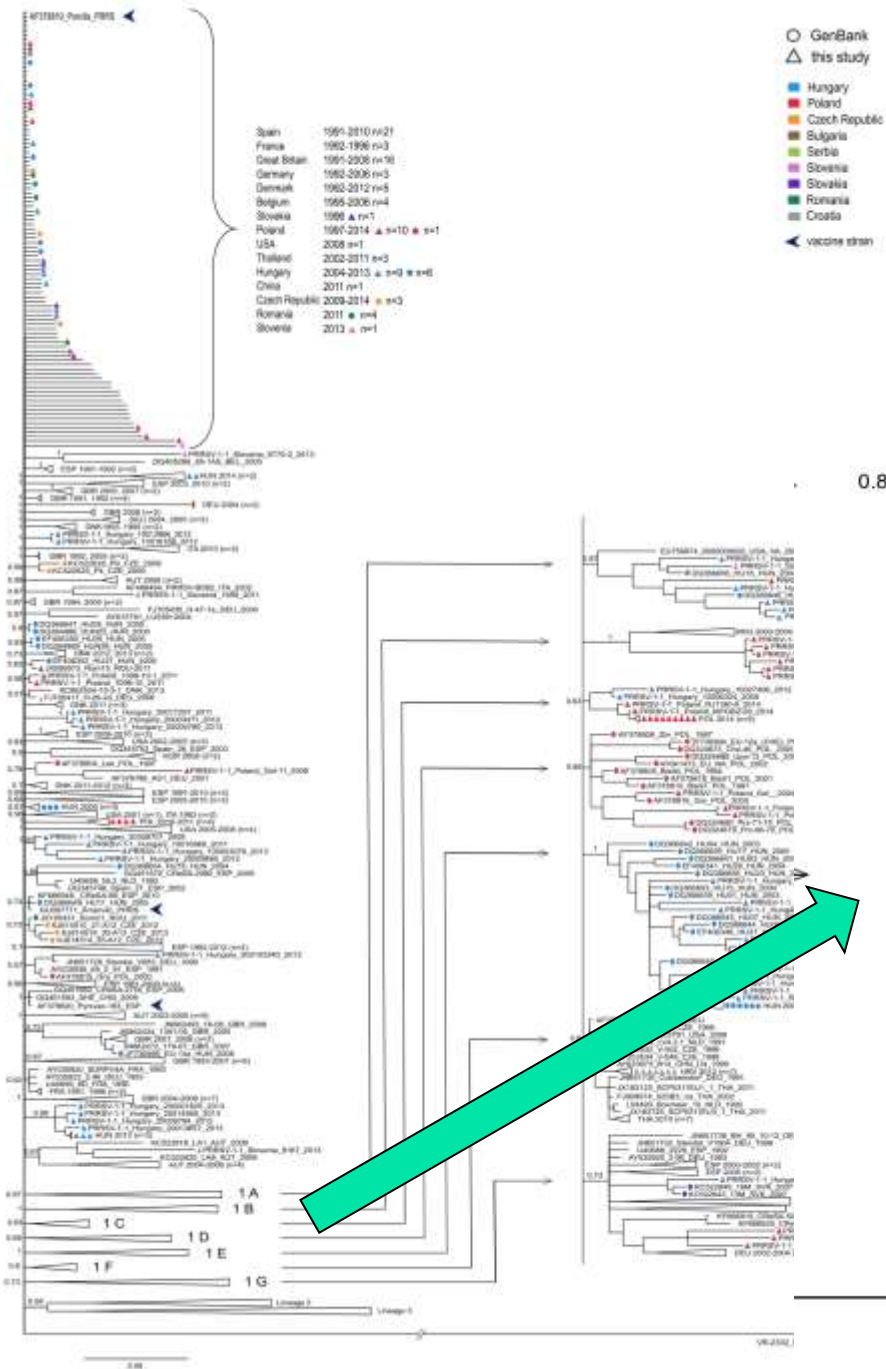
Genetic diversity of PRRSV 1 in Central Eastern Europe in 1994–2014: origin and evolution of the virus in the region

Gyula Balka¹, Katarzyna Podgórska², Manreetpal Singh Brar³, Ádám Bálint⁴, Daniel Cadar⁵, Vladimir Celer⁶, Lilla Dénes¹, Zuzana Dirbakova⁷, Anna Jedryczko⁸, Lázár Márton⁹, Dinko Novosel¹⁰, Tamaš Petrović¹¹, Ivo Sirakov¹², Dóra Szalay⁴, Ivan Toplak¹³, Frederick Chi-Ching Leung³ & Tomasz Stadejek¹⁴

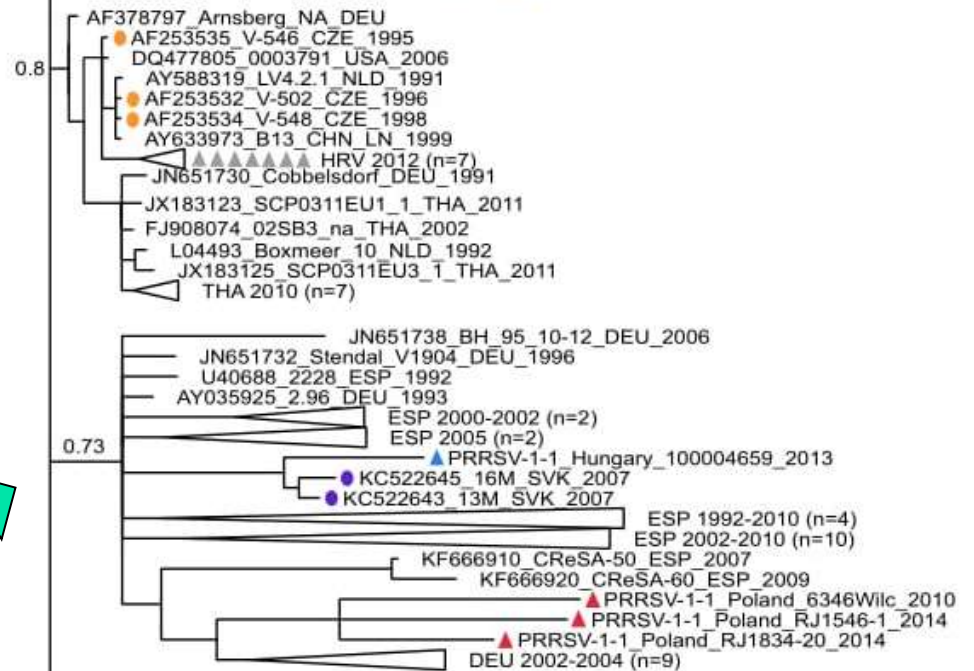
Received: 9 January 2018

Accepted: 1 May 2018

Published online: 17 May 2018



The origin of „Spanish like” strains in Central Eastern Europe

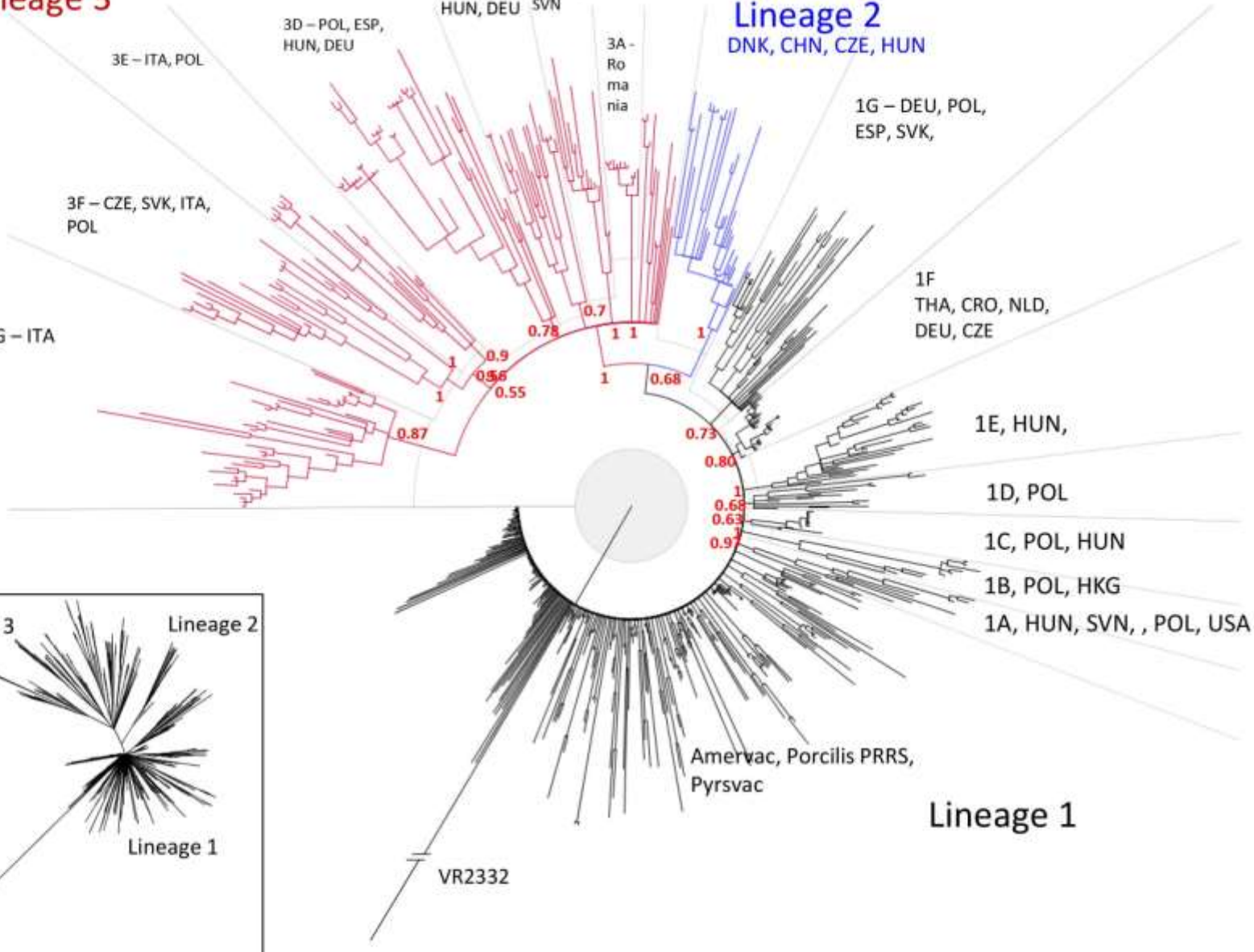
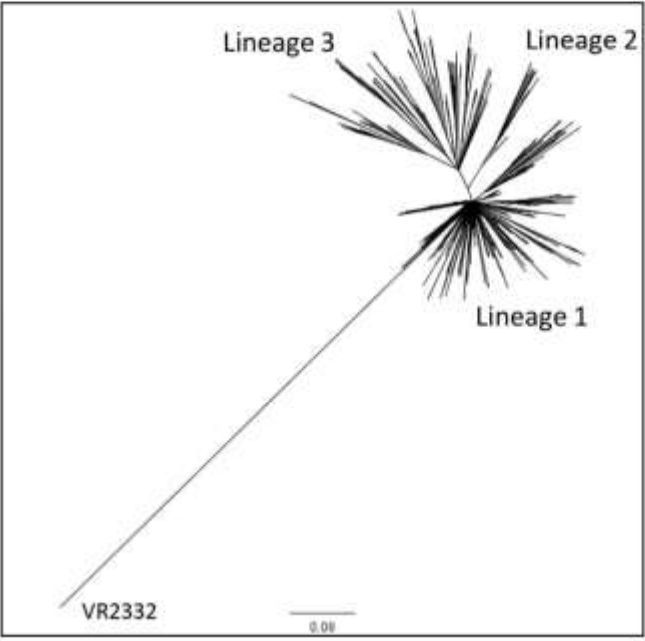


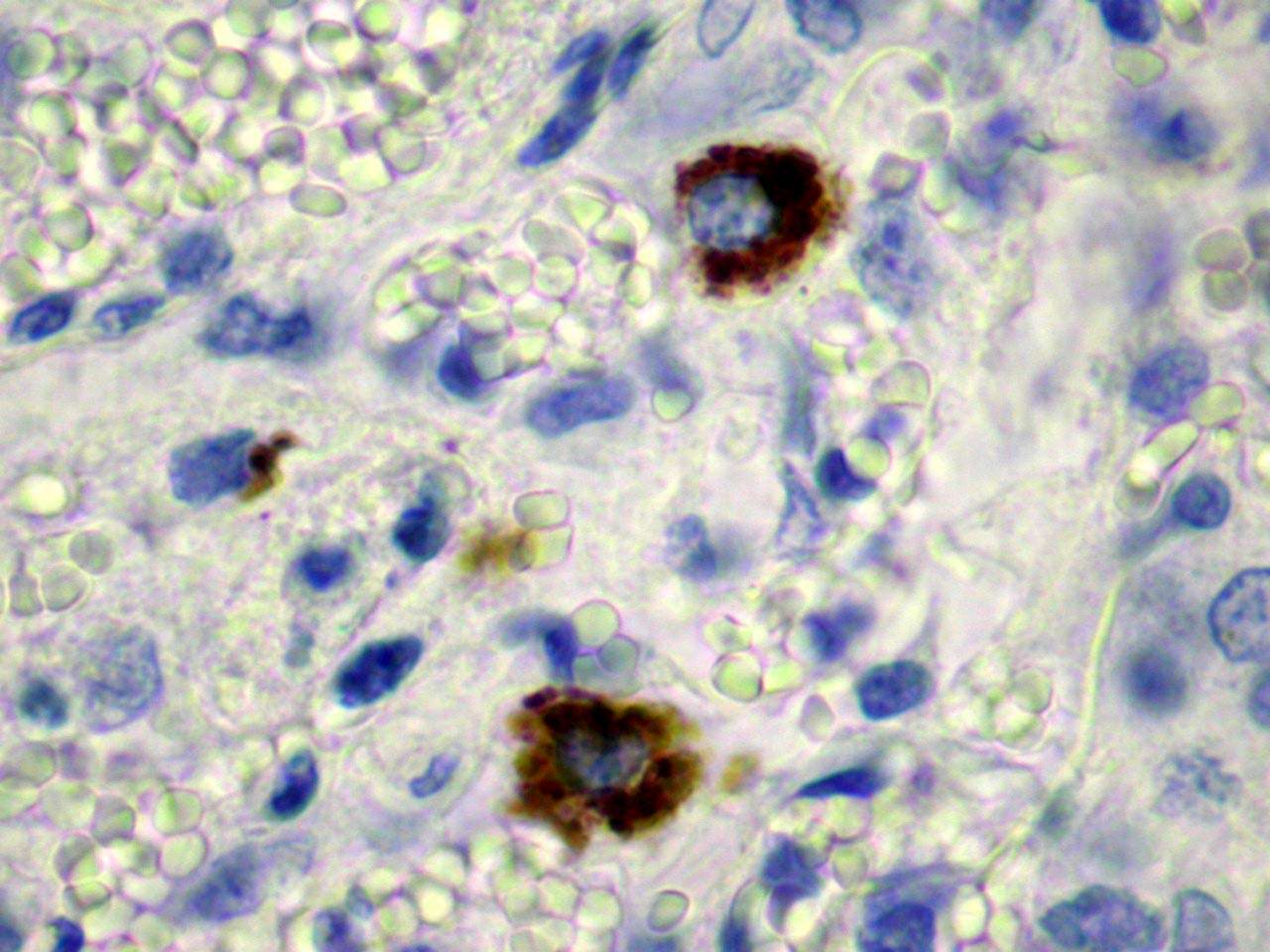
Lineage 3

3C - SRB, HUN, DEU
3B, ITA, SVN

Lineage 2

DNK, CHN, CZE, HUN



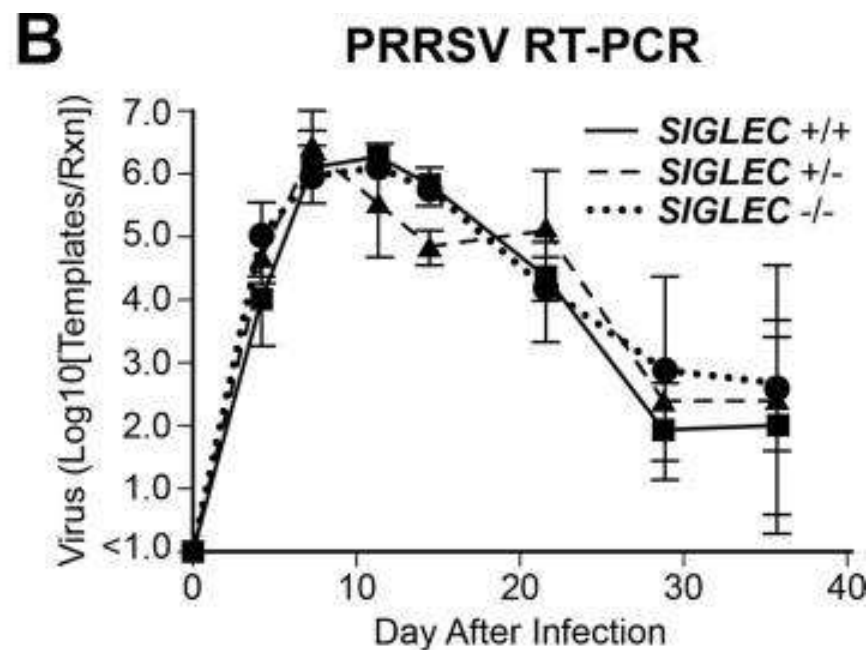
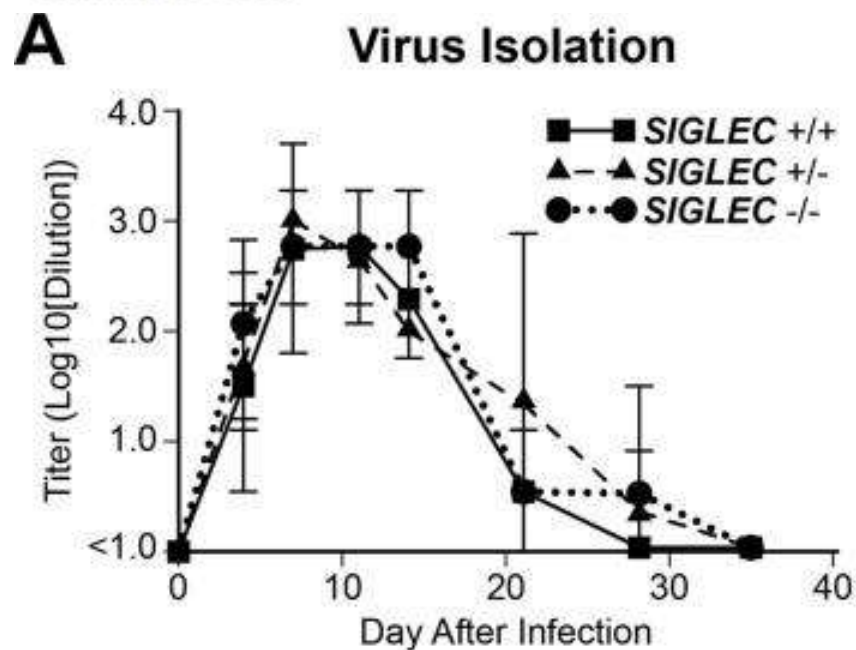


Disease resistance

- Genome editing of the host, knock out of the receptor for virus attachment
- Selection of pigs for disease resistance

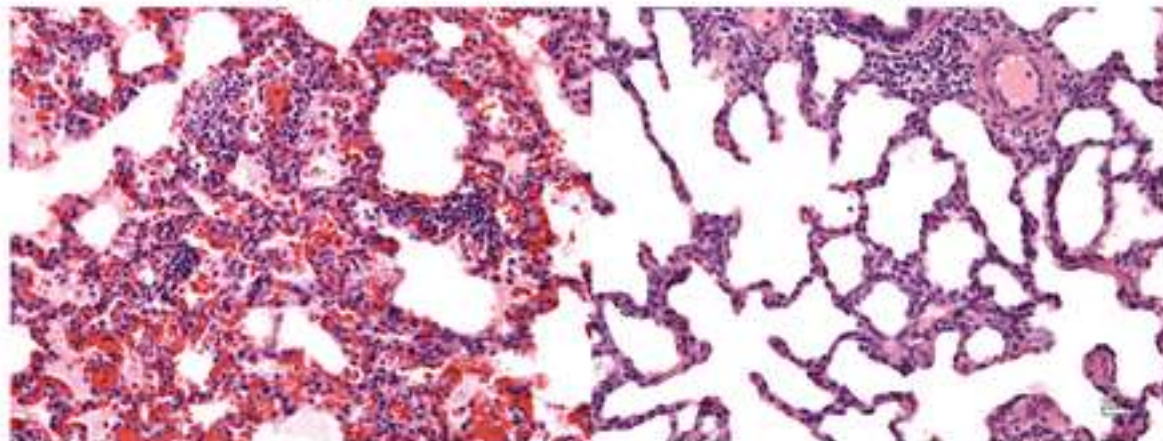
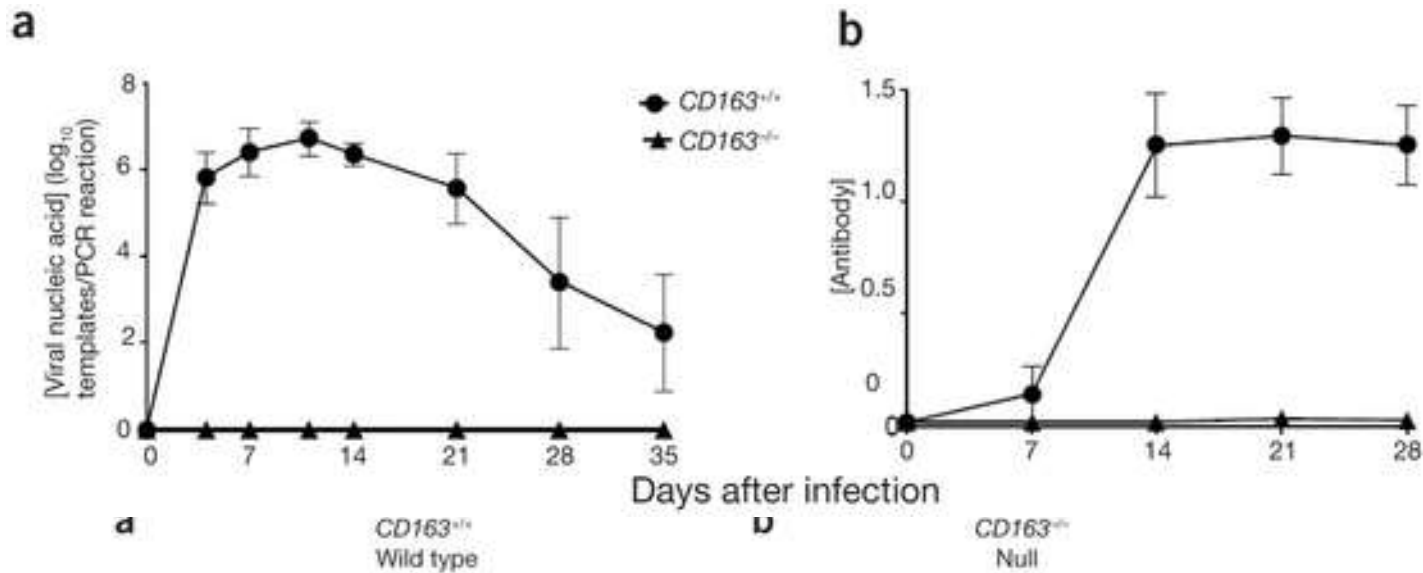
An Intact Sialoadhesin (Sn/SIGLEC1/CD169) Is Not Required for Attachment/Internalization of the Porcine Reproductive and Respiratory Syndrome Virus

Randall S. Prather,^a Raymond R. R. Rowland,^b Catherine Ewen,^b Benjamin Tribble,^b Maureen Kerrigan,^b Bhupinder Bawa,^b Jennifer M. Teson,^a Jiude Mao,^a Kiho Lee,^a Melissa S. Samuel,^a Kristin M. Whitworth,^a Clifton N. Murphy,^a Tina Egen,^a Jonathan A. Green^a



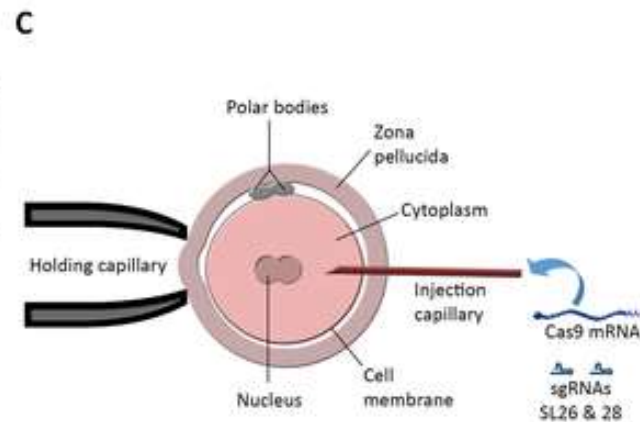
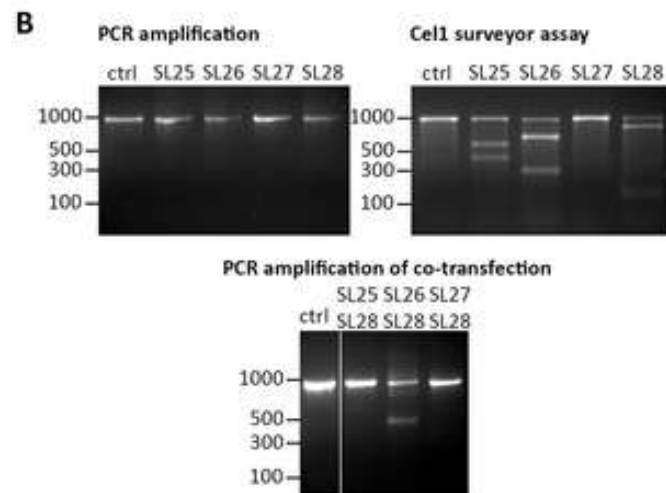
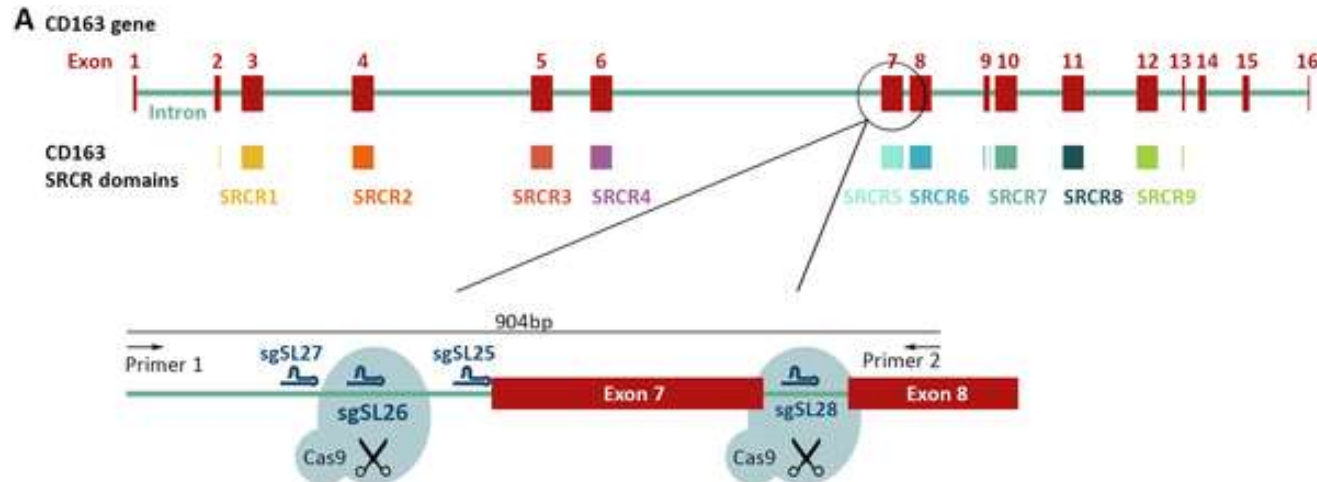
Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus

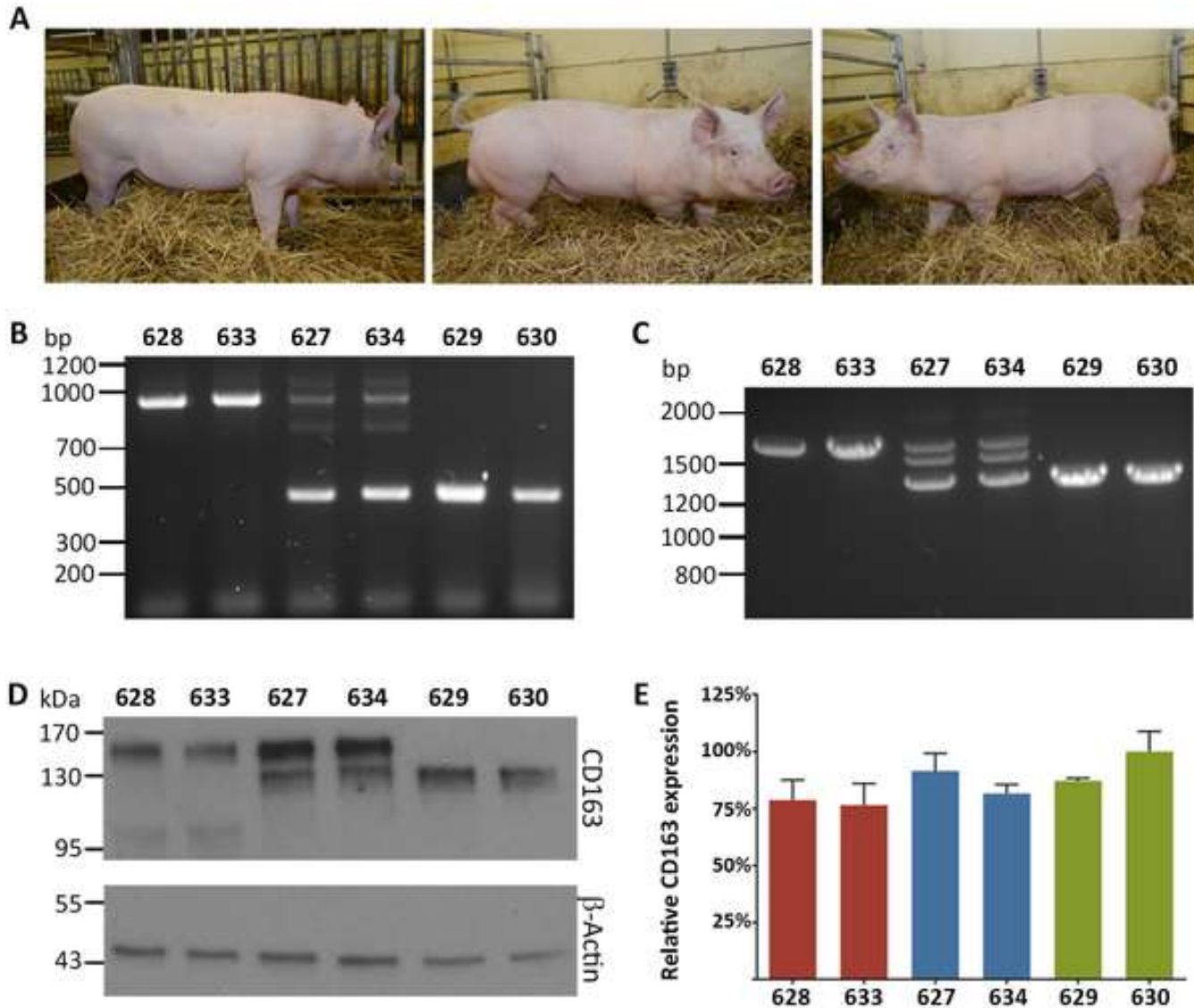
Whitworth KM, Rowland RR, Ewen CL, Tribble BR, Kerrigan MA, Cino-Ozuna AG, Samuel MS, Lightner JE, McLaren DG, Mileham AJ, Wells KD, Prather RS
Nat Biotechnol. 2016 Jan;34(1):20-2



Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function.

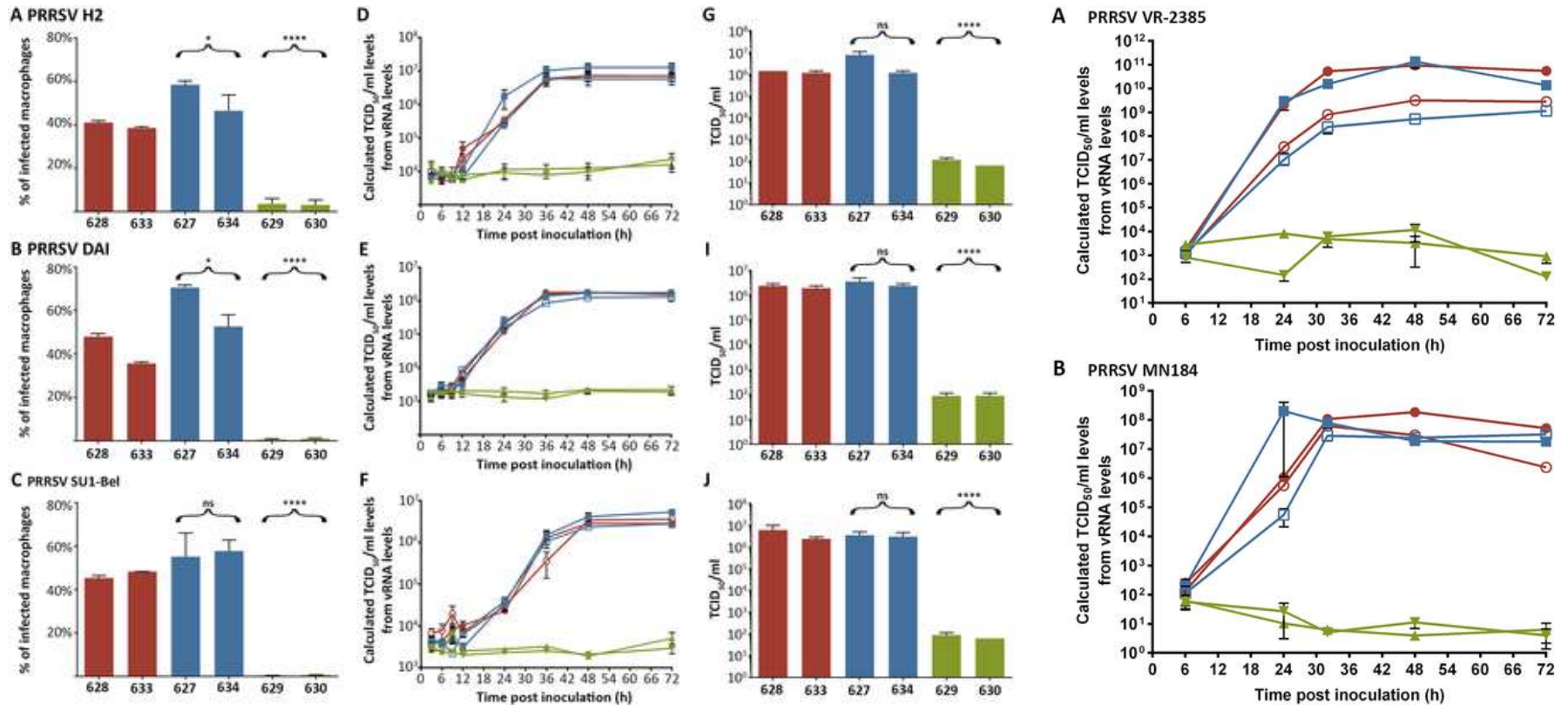
Burkard C et al. (2017) PLOS Pathogens 13(2): e1006206.





Burkard C et al. (2017) Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. *PLOS Pathogens* 13(2): e1006206.

ΔSRCR5 pulmonary alveolar macrophages (PAMs) are not susceptible to infection with PRRSV 1 and 2.



Burkard C et al. (2017) Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. PLOS Pathogens 13(2): e1006206.

What is disease resistance?

“The ability of the host to resist infection or exert control over the life cycle of the pathogen”

Resistance exists in different forms:

- 1.) Preventing infection upon exposure
- 2.) Limiting replication once infected

Resistance occurs at different levels:

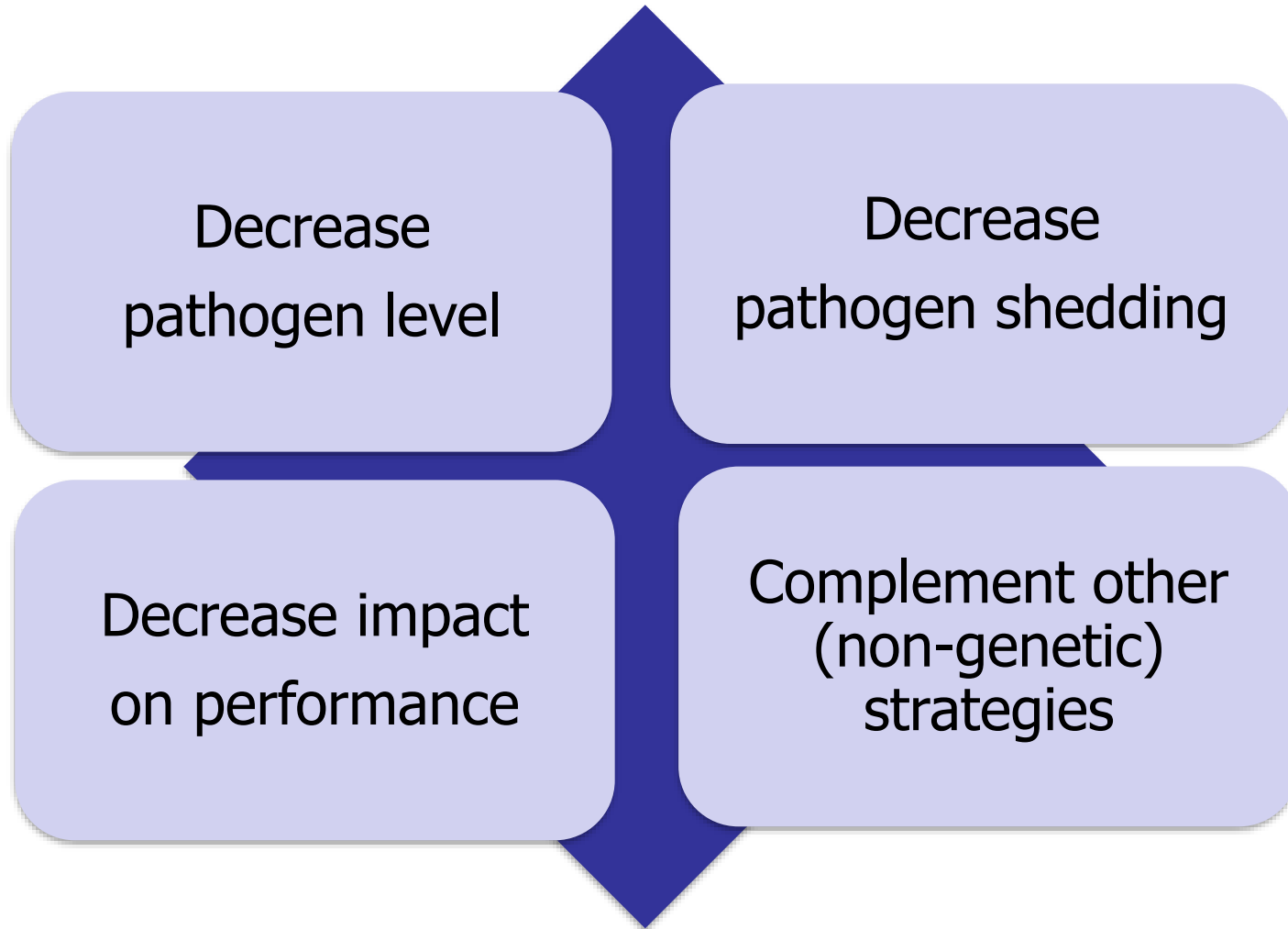
- 1.) Partial resistance
- 2.) Complete resistance



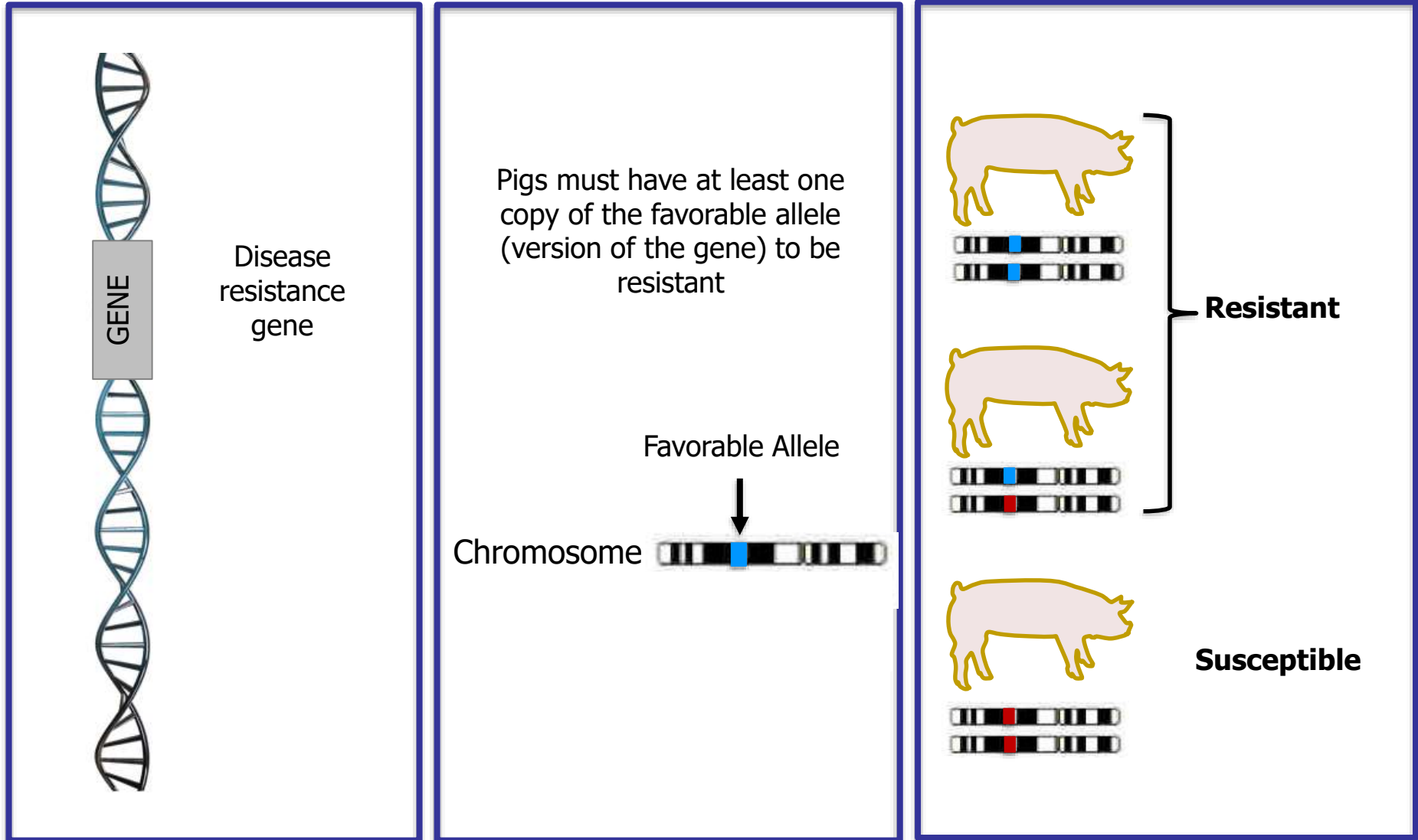
There is evidence of genetic variation in response to disease for nearly every disease intensively studied in livestock (Dr. Steve Bishop, 2014)

Why select for increased disease resistance?

Within a population, selecting for increased disease resistance can...



Selection for disease resistance: example

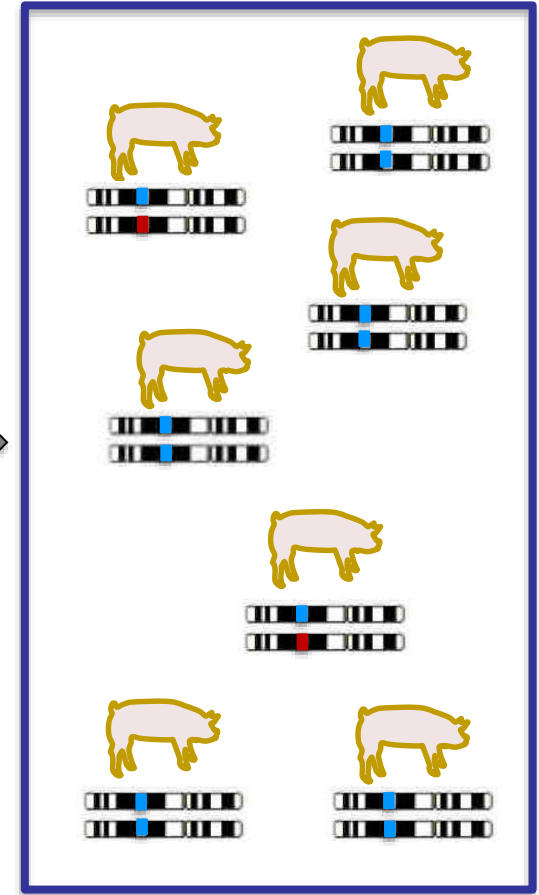
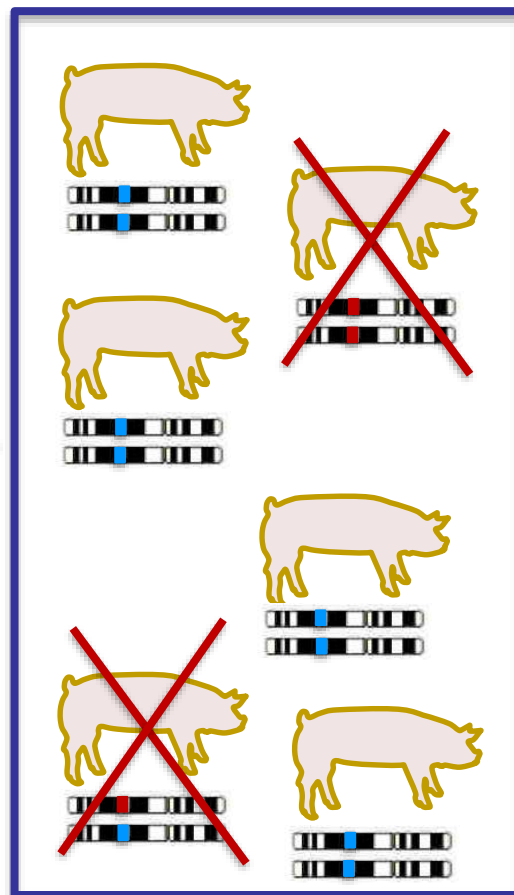
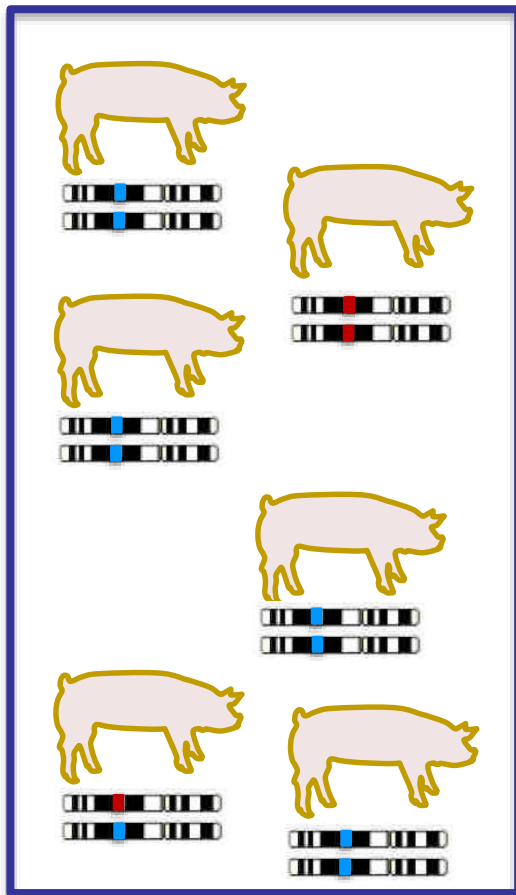


Selection for disease resistance: example

Male selection candidates are genotyped for the resistance gene

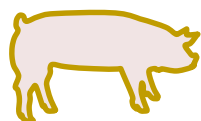
Only males with two copies of the favorable allele are used as parents

All progeny are resistant!

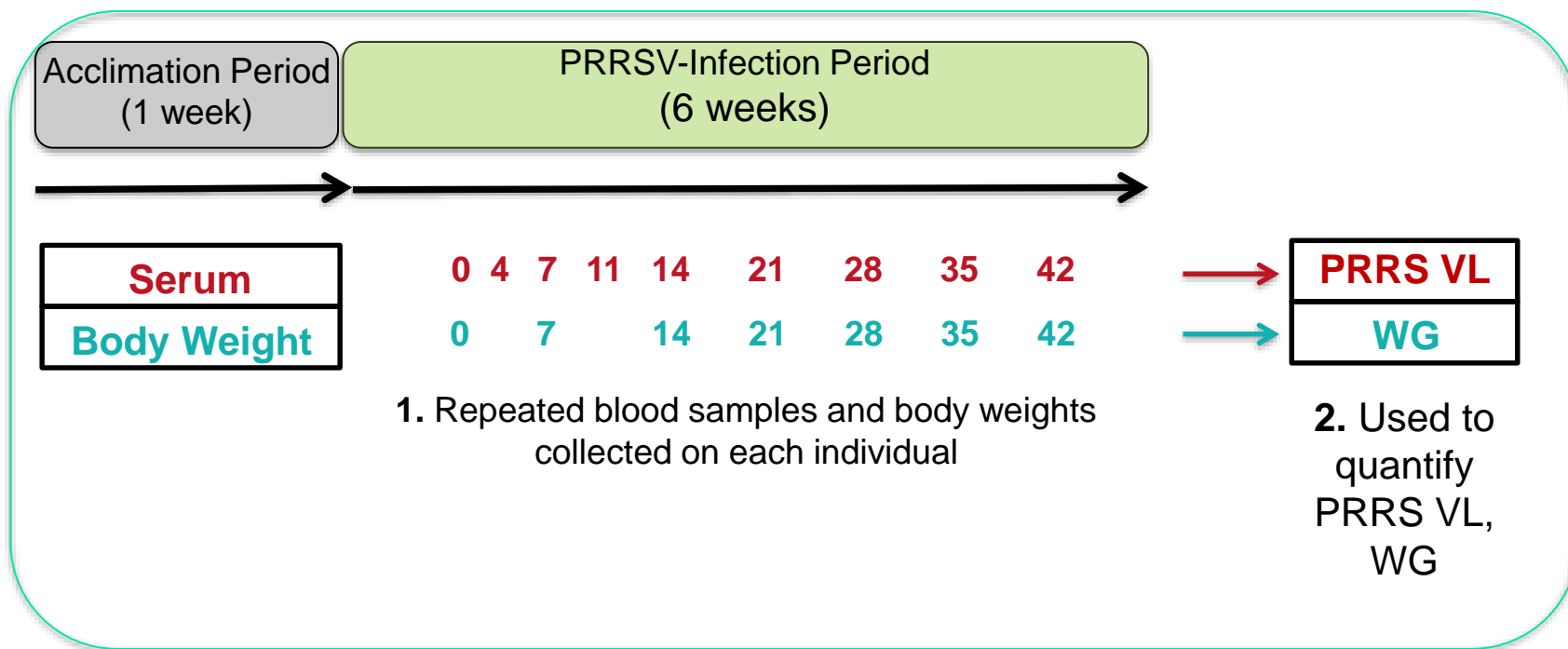


Design of PHGC trials

$n \sim 200$



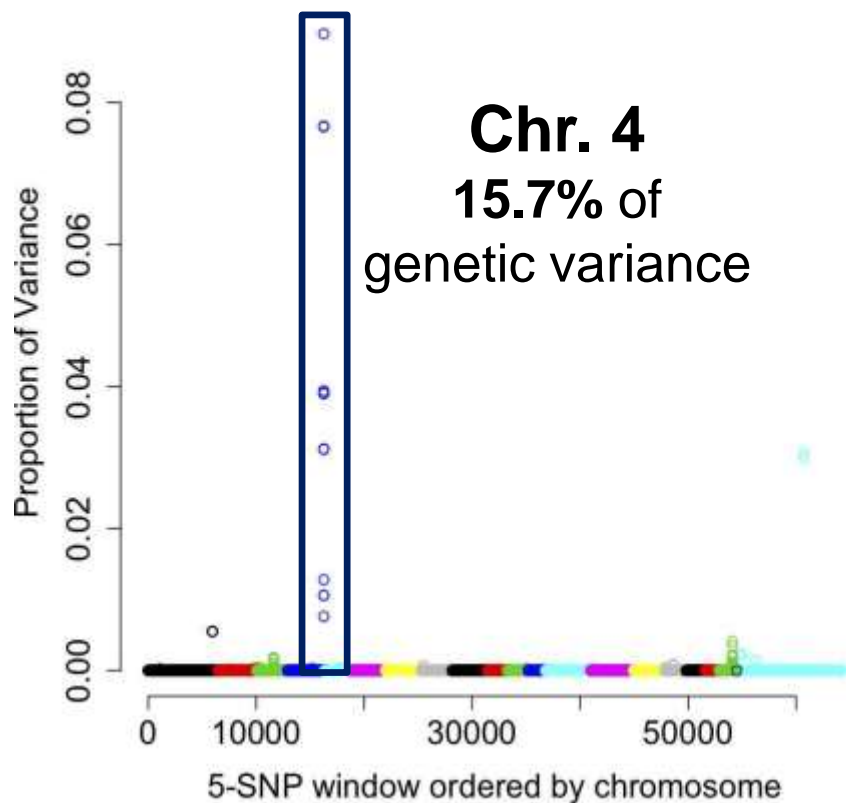
commercial crossbred nursery pigs per trial



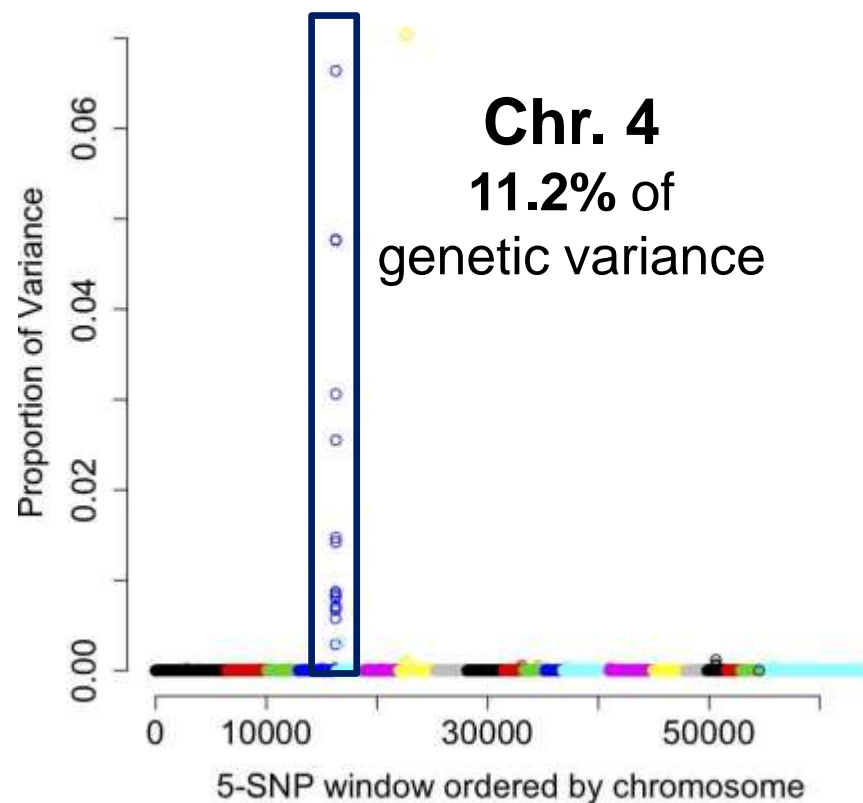
3.) Which genes/genomic regions are associated with disease resistance?

Genome-Wide Association Study (Boddicker et al., 2012)

PRRS Viral Load (VL)



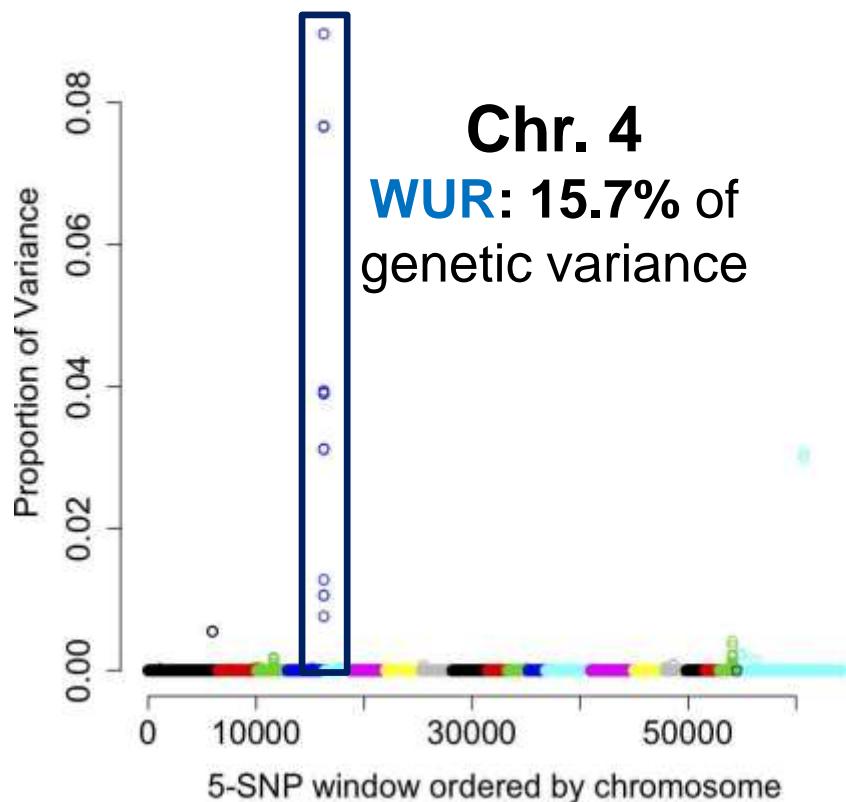
Weight Gain (WG)



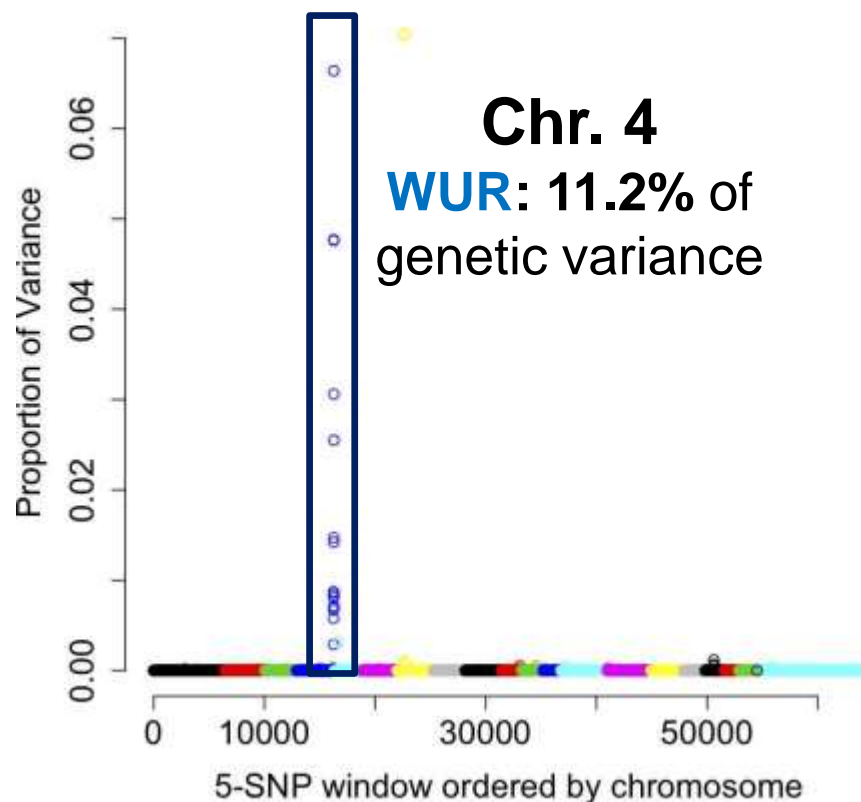
3.) Which genes/genomic regions are associated with disease resistance?

SNP WUR10000125 (WUR): genetic marker for the putative causative gene Guanylate Binding Protein 5 (**GBP5**) (Koltes et al., 2015)

PRRS Viral Load (VL)

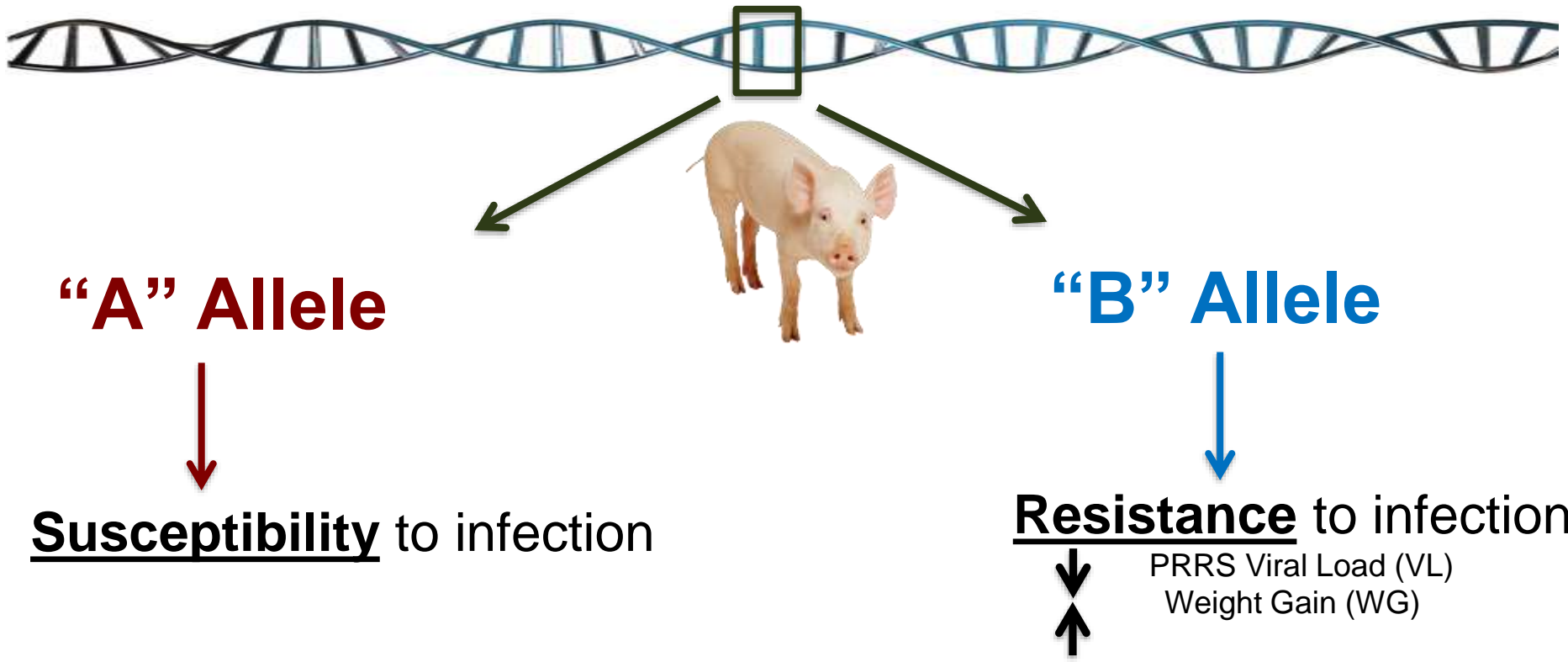


Weight Gain (WG)



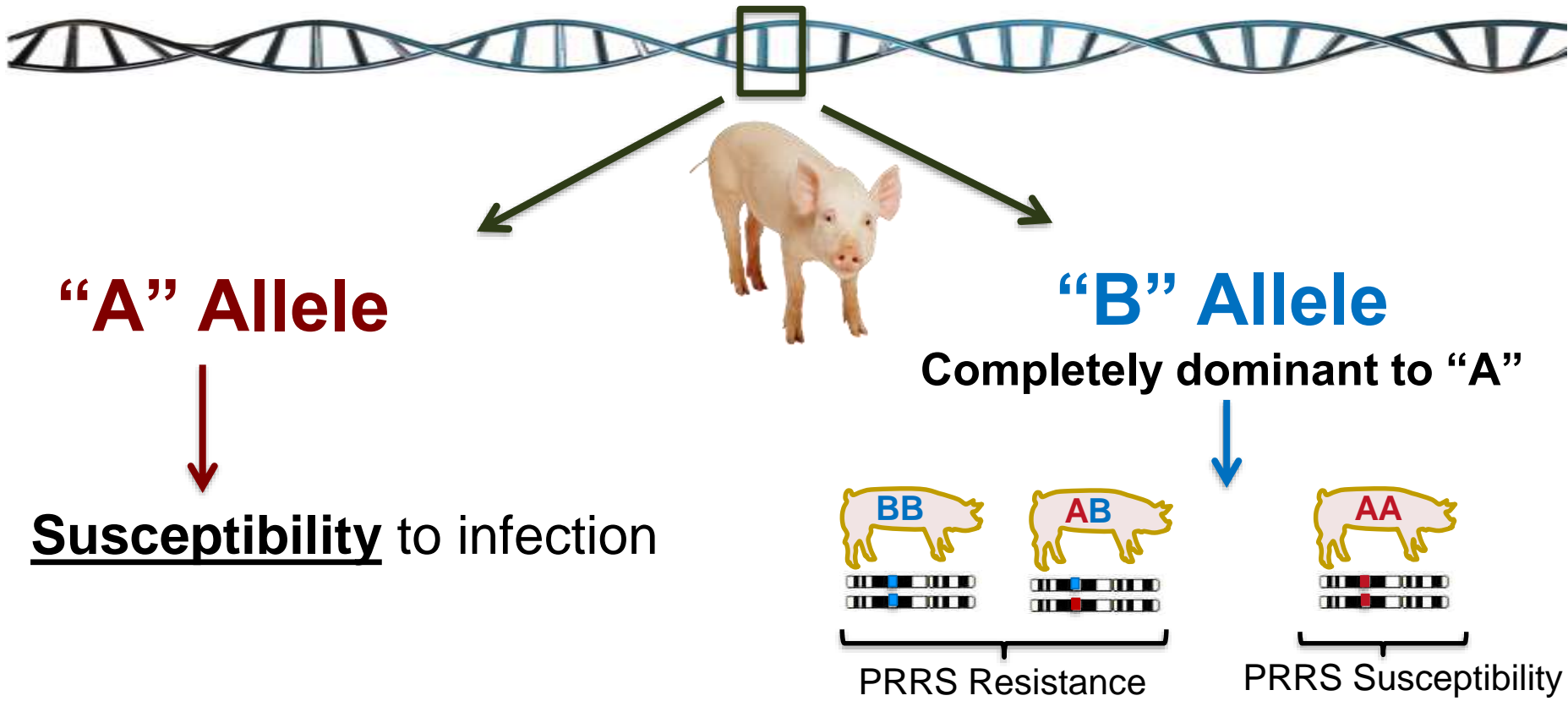
4.) What is the favorable allele for response to infection?

WUR Genotype: associated with host response to PRRS (Boddicker et al., 2012)



4.) What is the favorable allele for response to infection?

WUR Genotype: associated with host response to PRRS (Boddicker et al., 2012)

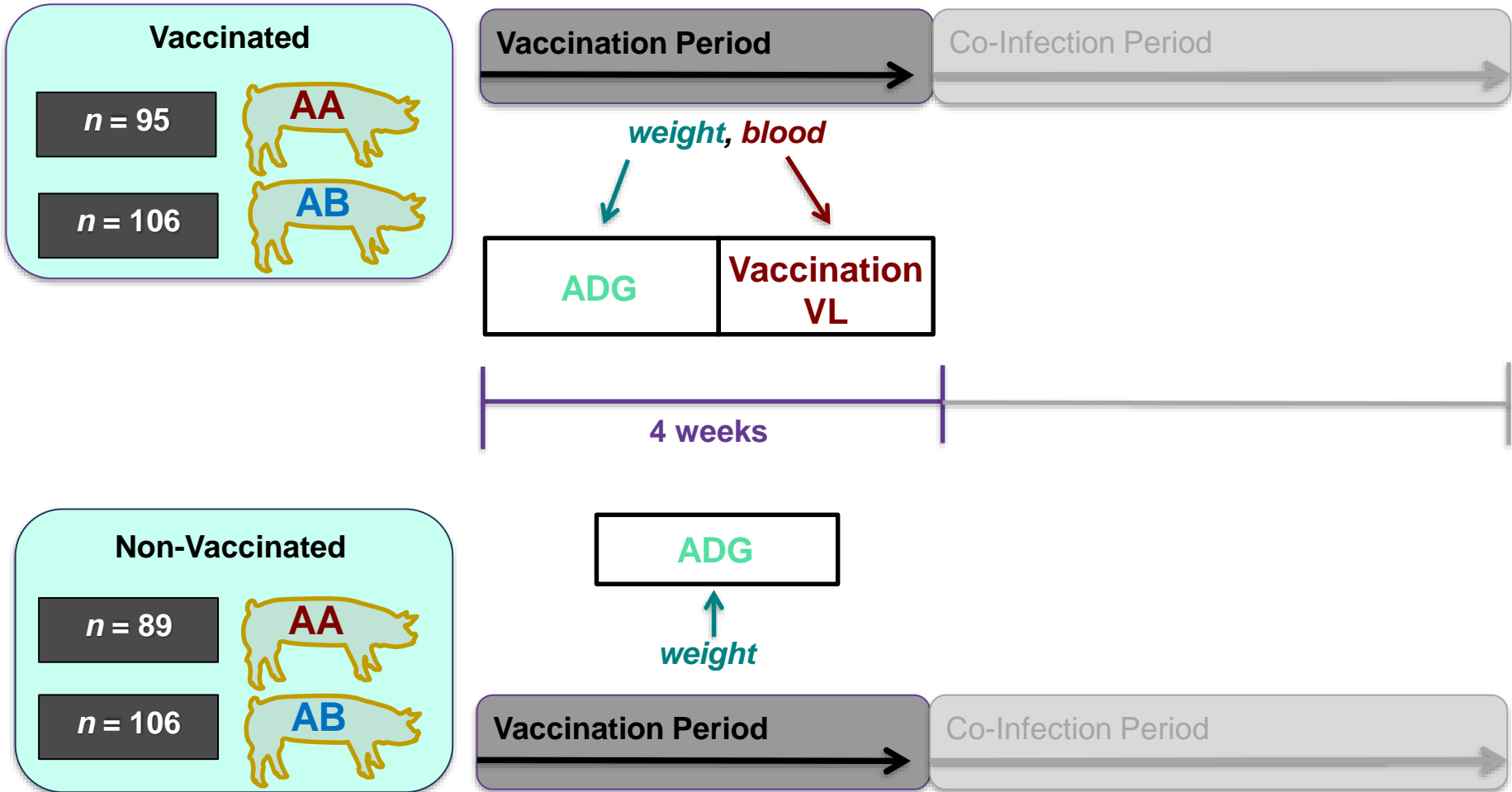


b.) Following vaccination for PRRS?

- Commercial vaccines becoming more available
- Modified live virus (**MLV**) vaccines
 - Most effective type
- “Modified” → different type of PRRSV challenge

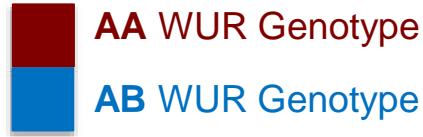


b.) Following vaccination for PRRS?

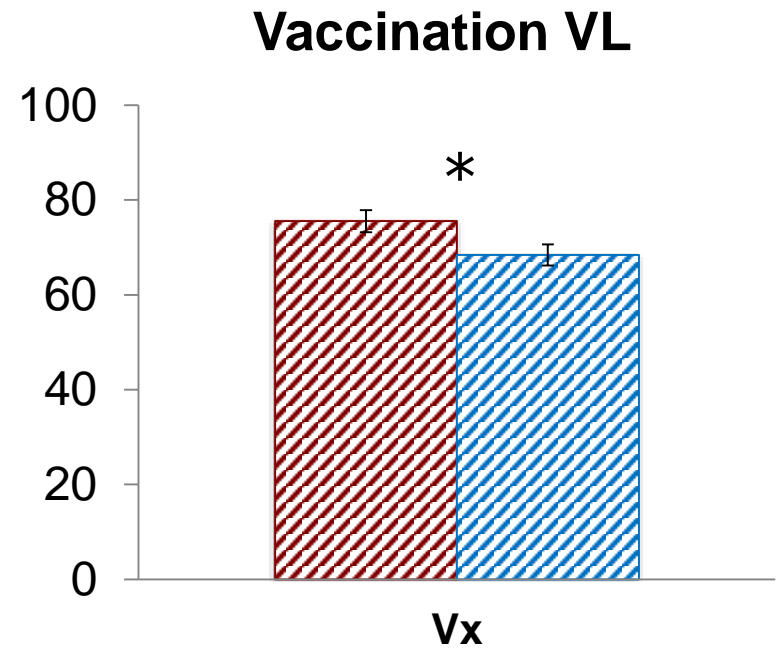
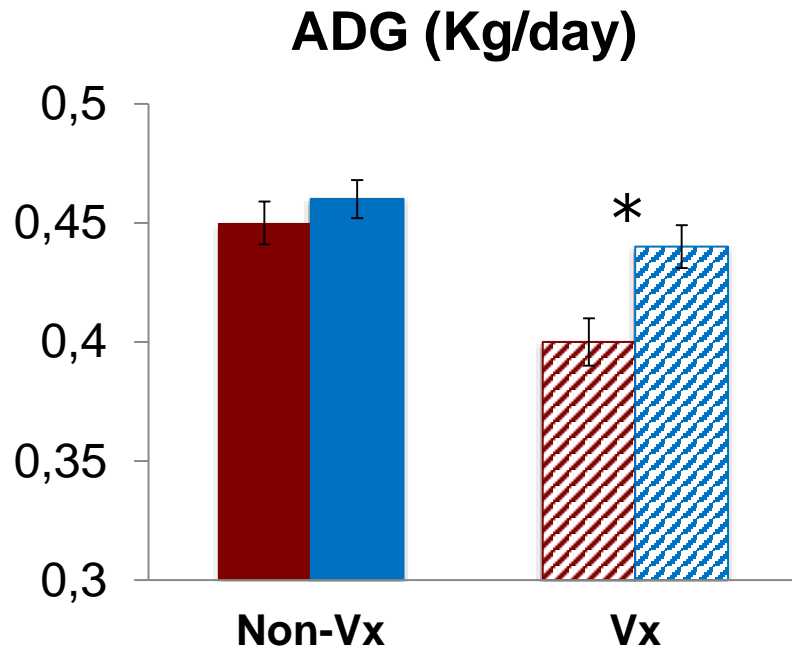




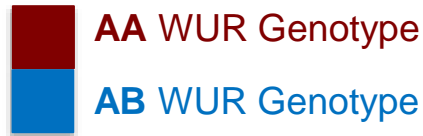
b.) Following vaccination for PRRS?



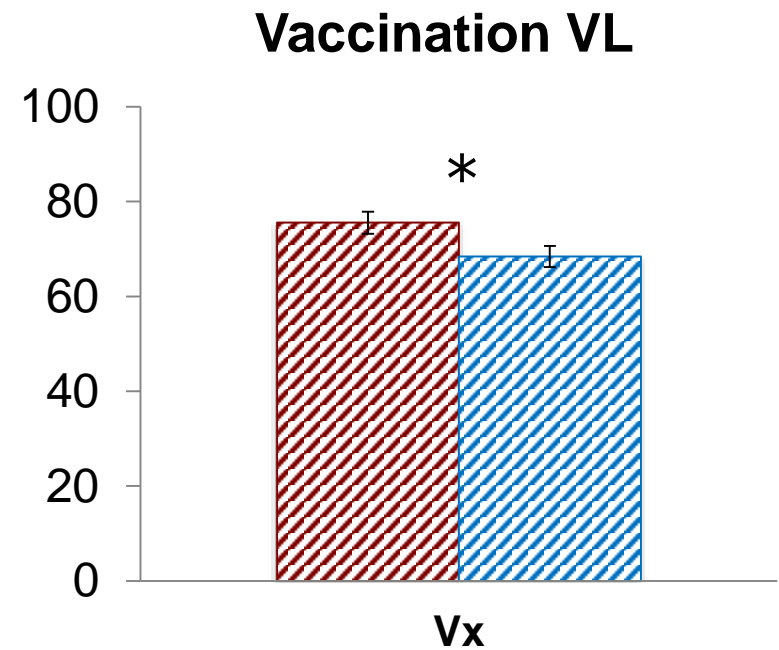
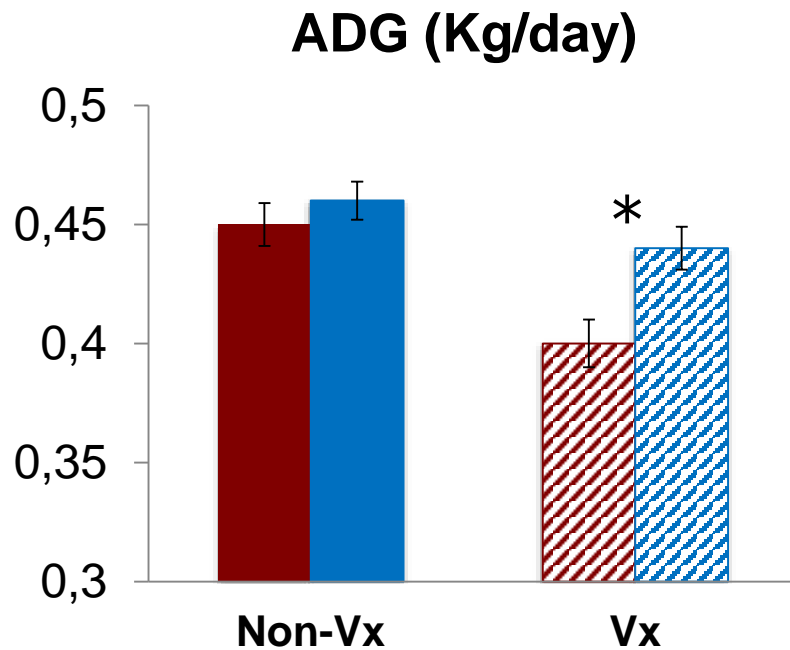
* P < 0.05
** P < 0.001



b.) Following vaccination for PRRS?



* P < 0.05
** P < 0.001



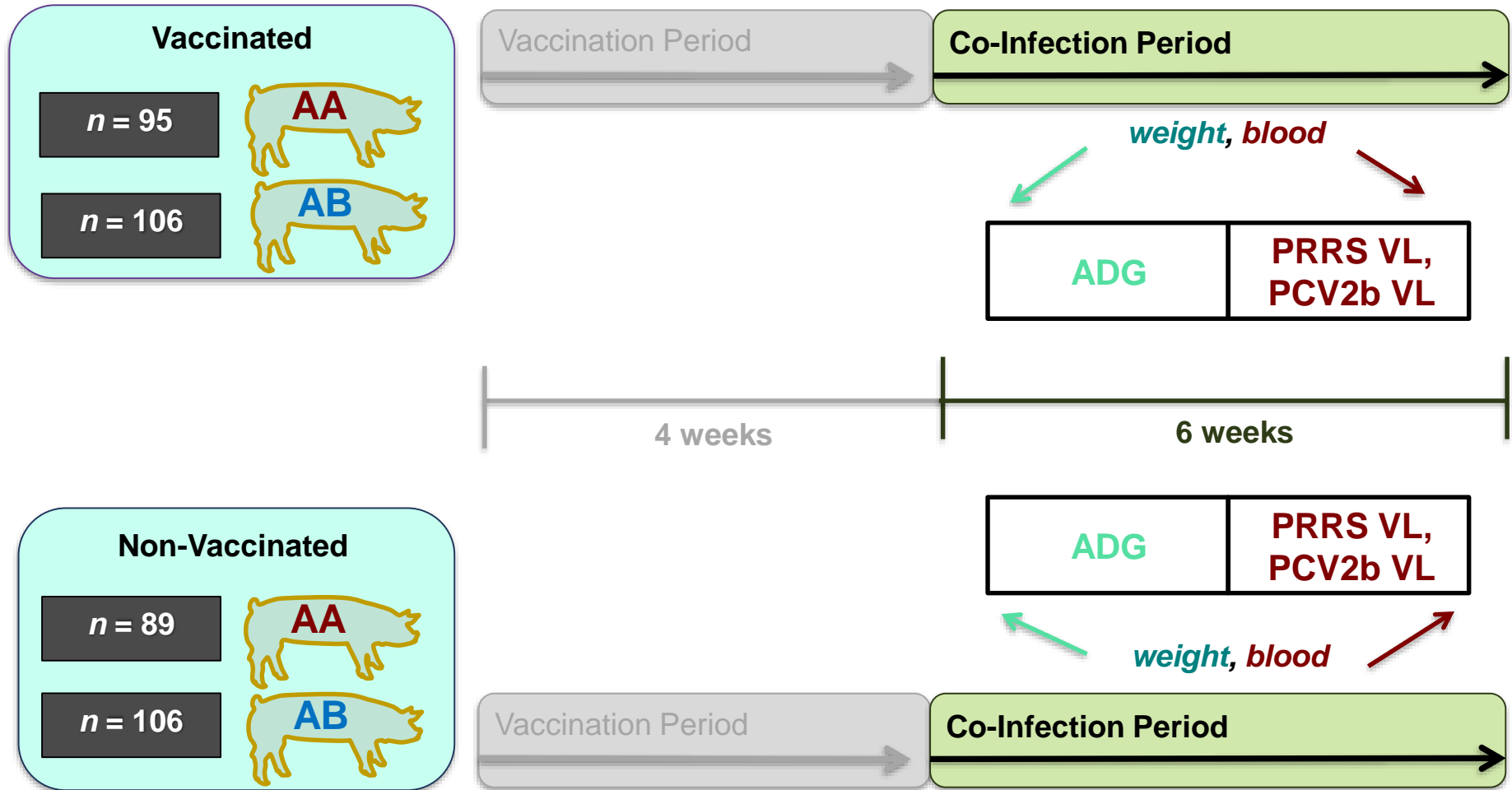
The B allele is favorable following PRRS vaccination:
Associated with: **↑** ADG & **↓** Vaccination VL

A WUR SNP is associated with European Porcine Reproductive and Respiratory Virus Syndrome resistance and growth performance in pigs

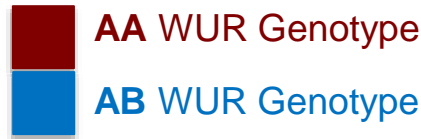
Abella et al. 2016, Res. Vet. Sci.

- There is variation in the virus load in challenged pigs with a European PRRSV strain.
- A WUR SNP is associated with growth rate in pigs challenged with an attenuated European PRRSV strain.
- The AG pigs perform better than the AA pigs in PRRSV infected animals.
- The AA pigs show a better performance than the AG pigs in a PRRSV-free environment.
- Non-viremic pigs will not become a reservoir for an attenuated European PRRSV strain in tonsil.

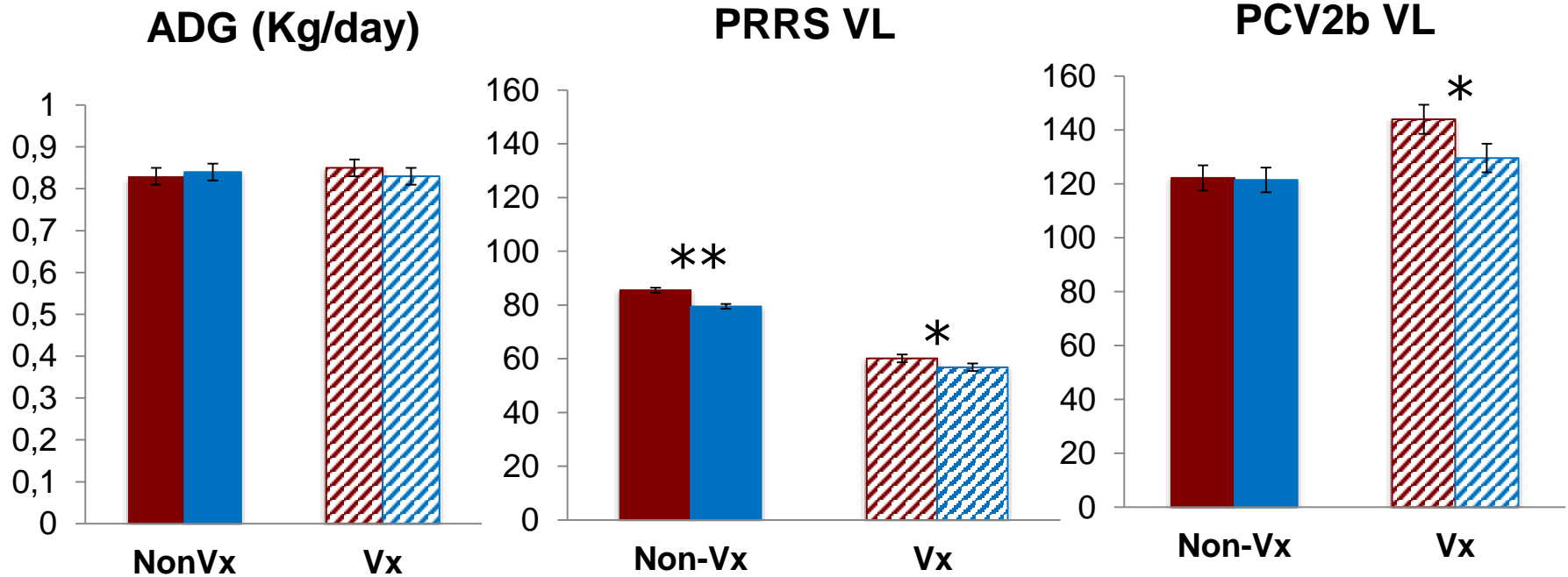
c.) Following co-infection with other pathogens?



c.) Following co-infection with other pathogens?



* P < 0.05
** P < 0.001



The **B** allele is favorable following PRRSV/PCV2b co-infection:

Associated with: ↓ PRRS VL & ↓ PCV2b VL (when previously vaccinated for PRRS)

The disease - diagnostics

Economic impact of PRRS

„The most costly disease of swine production worldwide”
(Chand et al. 2012)

- Neumann et al. 2005 – 560 M USD, 12% – 88%
- Holtkamp et al. 2012 – 664 M USD, 45% – 55%

- USA (Holck and Polson, 2003)
 - 255 USD/sow
 - 6,25–15-25/growin pig
- The Netherlands (Nieuwenhuis, 2012)
 - 75 (59–379) EUR
- Denmark (Kristensen, 2012)
 - Acute PRRS: 31 (5–100) EUR
 - Endemic PRRS: non significant
 - Slight increase in mortality
 - No increase in AB use



Stages of PPRSV infection in the pig

1. **acute phase** – lung tissue and upper respiratory tract (Mø and DC)
 - Viraemia up to weeks in young animals
2. **persisting phase** – lymph nodes, tonsil
 - No viraemia, not in the lungs, no clinical signs, BUT virus can be transmitted to naïve pigs
3. **elimination phase** - max. 250 days PI: lifelong infection in growing pigs
 - The basis of herd closure and roll over method

PRRS cycle in the herd

Introduction in the herd

(SOWS)

Clinical phase
(≈ 2 months)

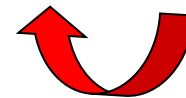
Congenitally infected viraemic piglets

Seroconversion
(more than 90%)

Elimination?

Minimal virus circulation

Virus introduction in the
nursery



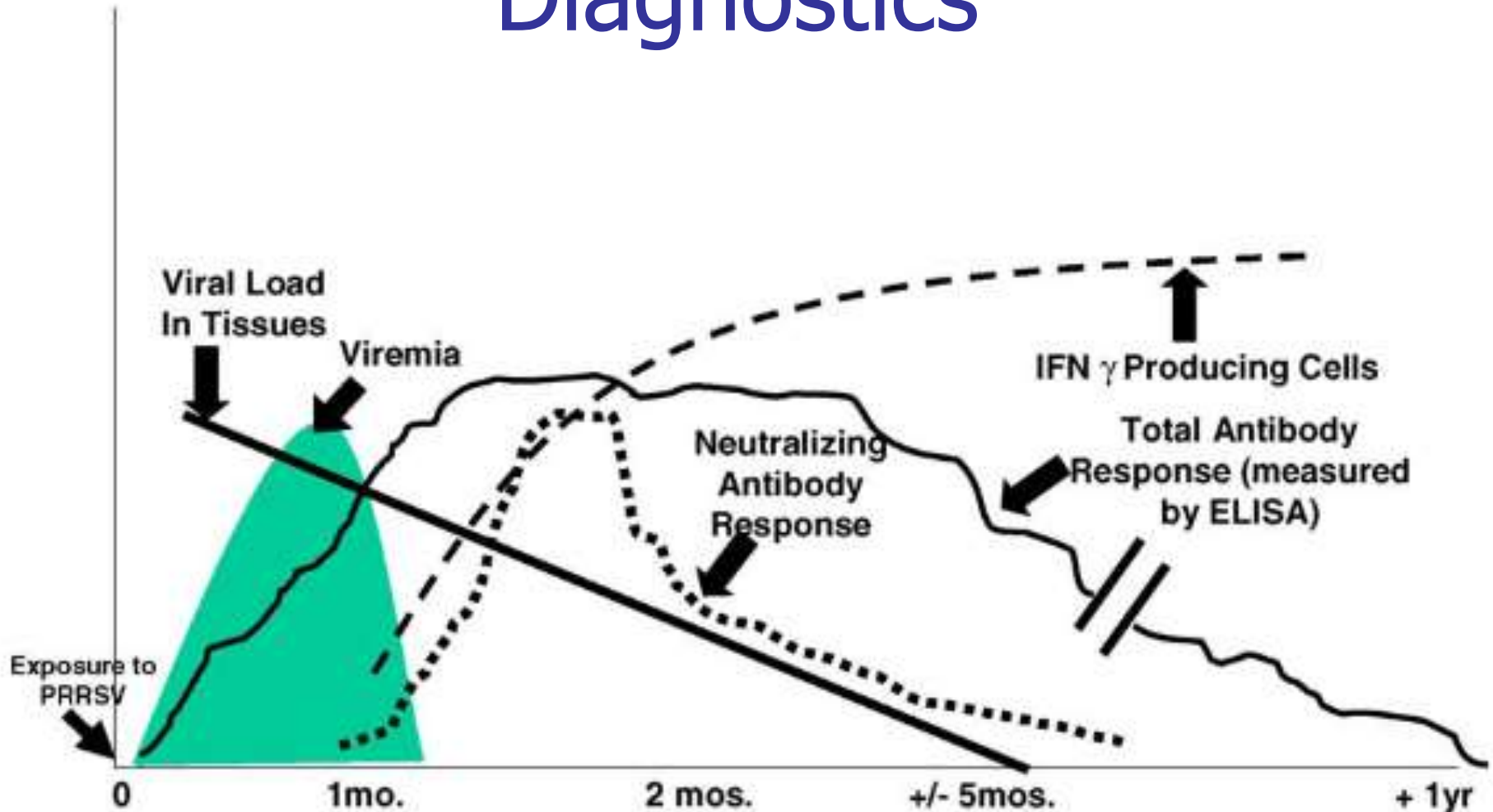
Endemic infection

**Susceptibility will increase
over time**

(loss of immunity, naïve replacement
gilts etc.)

New outbreak
(~1,5–2 years)

Diagnostocs



Lopez and Osorio, 2004

Diagnostics

- **Sensitivity**: the probability that positive samples the test detects are truly positive
 - Decreased sensitivity: more false negatives
- **Specificity**: the probability that negative samples the test detects are truly negative
 - Decreased specificity: more false positives
- ELSIA, PCR, qPCR

False positive paradoxon: 95% specific test

40%

Number of people	Infected	Uninfected	Total
Test positive	400 (true positive)	30 (false positive)	430
Test negative	0 (false negative)	570 (true negative)	570
Total	400	600	1000

93%

2%

Number of people	Infected	Uninfected	Total
Test positive	20 (true positive)	49 (false positive)	69
Test negative	0 (false negative)	931 (true negative)	941
Total	20	980	1000

29%

Diagnostics

	Clinical diagnosis	Seroprofilng of herds	ERADICATION	Monitoring negative status	Controlling vaccination
Prevalence	0–100%	0–100%	100 → 0%	0%	0–100%
Specificity	+	+	+	++	+
Sensitivity	++	+	++	+	+

Erik van Esch, EUROPRRS 2012

Different diagnostic goals

- Find the virus in an outbreak
 - PCR, ELISA – target the diseased group, relative low number could be sufficient, sequencing of positives
 - Piglets, respiratory tract, lung + ln, thymus from stillborns, serum of piglets – PCR, ELISA
 - Serum of sows – ELISA
- Cross sectional profiling of a positive herd
 - ELISA, PCR – every age group needs to be tested
 - Serum, oral fluid

Different diagnostic goals

- Catch the virus in a subclinical infection
 - PCR
 - Lung + In.
 - Serum

Age groups	Virus prevalence					Sero-prevalence				
	<i>n</i>	Mean (%)	95% confidence interval (%)	Sample size (<i>n</i>)	95% confidence interval sample size (<i>n</i>)	<i>n</i>	Mean (%)	95% confidence interval (%)	Sample size (<i>n</i>)	95% confidence interval sample size (<i>n</i>)
Sows	185	0.5	0-1.6	450	170-1000	40	53	37-69	7	3-11
Piglets 9 weeks	92	30	21-40	9	6-13	178	29	23-35	15	12-19
Fatteners/breeding stock 16 weeks	120	27	19-35	10	7-15	180	61	54-68	6	3-7
Fatteners/breeding stock 22 weeks	97	8	3-14	36	20-94	180	69	62-76	3	3-6

Different diagnostic goals, PRRS

■ Eradication

- The prevalence decreases, sample size has to be increased
- More samples will increase the risk of false positives
- Very sensitive test is needed – increased amount of false positives
- Costs are increasing

Different diagnostic goals

- Eradication – what can we do??
 - Scientifically chosen sample numbers, confidence intervals, estimated prevalence
 - Choosing the right group of animals: sentinel gilts after herd closure, piglet sampling to check sow herd stability
 - Serum samples – ELISA in sows, PCR in piglet sera (pooling), (PCR in case of carcasses)

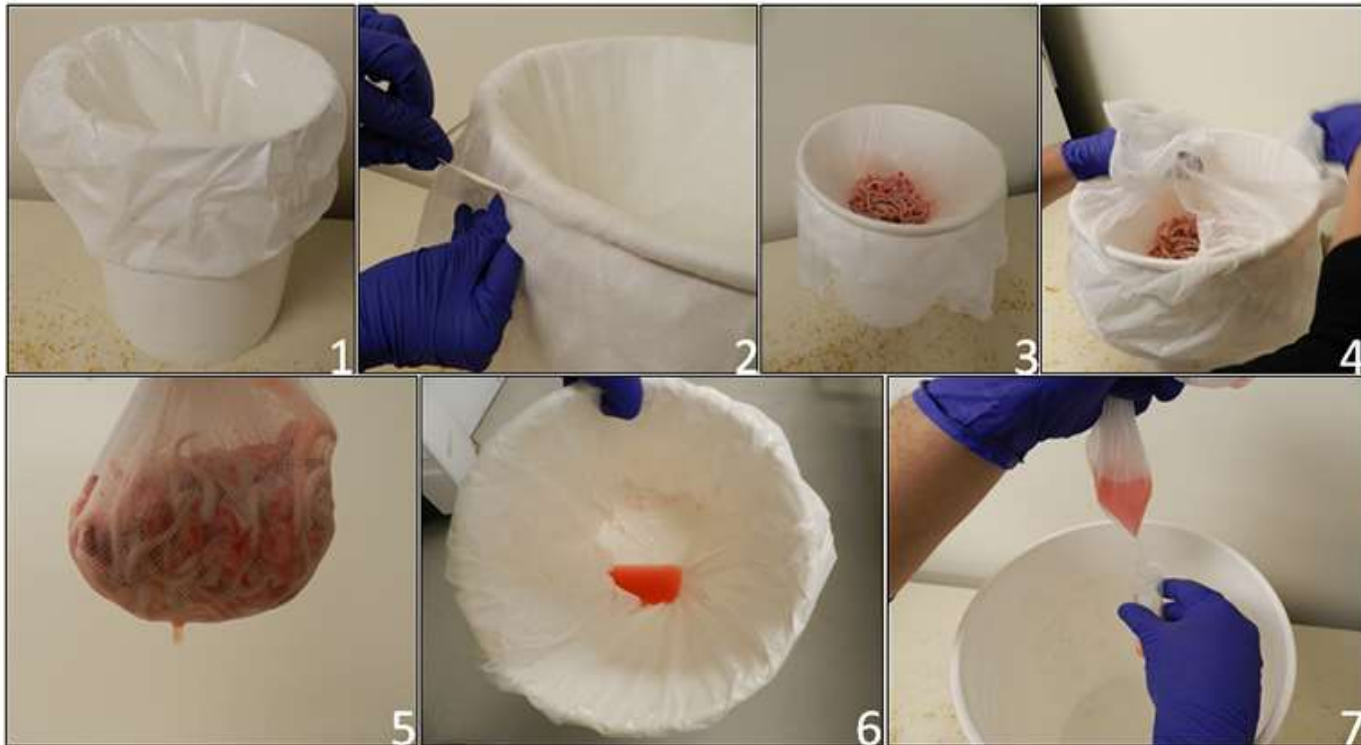
		Population Size (Detecting One or More Positives)			
Prevalence Estimate	Confidence Level	100	1000	4000	10000
		>1%	70%	71	114
	80%	81	149	158	160
	90%	91	206	224	228
	95%	96	259	289	295
	99%	100	369	434	449
>10%	70%	12	13	13	13
	80%	16	17	17	17
	90%	21	23	23	23
	95%	26	30	30	30
	99%	37	44	45	45
>25%	70%	6	6	6	6
	80%	7	7	7	7
	90%	9	10	10	10
	95%	11	12	12	12
	99%	16	17	18	18
>50%	70%	3	3	3	3
	80%	4	4	4	4
	90%	5	5	5	5
	95%	6	6	6	6
	99%	8	8	8	8

Different diagnostic goals

- Eradication – what can we do??
 - Scientifically chosen sample numbers, confidence intervals, estimated prevalence
 - Choosing the right group of animals: sentinel gilts after herd closure, piglet sampling to check sow herd stability
 - Serum samples – ELISA in sows, PCR in piglet sera (pooling), (PCR in case of carcasses)
 - Aggregate samples

Aggregate samples

- Oral fluid
- Processing fluid



Different diagnostic goals

- Monitoring freedom of disease
- After eradication, or commercial purposes (selling boars, semen, gilts)
 - Well estimated number of animals
 - Sometimes individual testing – boars, gilts
 - ELISA, (PCR for semen – very fastidious, not reliable, false negatives due to inhibitors)

**Thank you for your
attention!**

